QUARTERLY JOURNAL

OF

MICROSCOPICAL SCIENCE.

VOLUME 80. New Series.
WITH PLATES AND TEXT-FIGURES

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The Posterior End of Meckel's Cartilage and related ossifications in Bony Fishes.

By

R. Wheeler Haines.

With 16 Text-figures.

INTRODUCTION.

IT has been shown recently (Haines, 1934) that the branchial bones of fishes grow by a typical epiphysial mechanism similar to that found in the long bones of tetrapods. The cartilages contain typical zones of undifferentiated, flattened, and hypertrophied cells, and the hypertrophied cells are eroded and replaced by endochondral bone and marrow. Separate epiphysial centres of ossification were not found, but in Sciaena a secondary centre of calcification was seen within the epiphysial cartilage.

This work was followed by a search among the other parts of the skeleton of fishes for epiphysial structures, and it was found that they are rather uncommon. On the other hand, it was noticed that the cartilages of the palatopterygoid, quadrate, and mandibular regions often showed very clear evidence of differentiation into zones of unspecialized, flattened, and hypertrophied cells, but that these zones were not arranged as they are in epiphyses. The posterior end of the mandible was chosen for special study as it could be removed from a museum specimen without greatly disfiguring it, and could be orientated easily for longitudinal section.

Some difficulty has been found in the terminology of the various kinds of bones, and of the tissues in which they are developed. In primitive fishes the dermal or membrane bones and the cartilage bones are well separated, the first group being developed in the dermis and connective tissues, and the second group in the perichondrium and cartilage. In later fishes both kinds of bone have spread into the connective tissues between the dermis and perichondrium. In some cases a cartilage bone

may reach the dermis as in the retro-articular bone of teleosts, or a membrane bone which was originally dermal in position may invade the perichondrium and cartilage as in the angular of the more specialized teleosts. But whatever the original nature of the bone it is often convenient to speak of membranous, perichondral, and endochondral parts of a bone in any individual fish. The dermal bones in primitive fishes would be described as being wholly membranous in composition, and the cartilage bones as having perichondral and endochondral parts, but in later fishes both dermal and cartilage bones may be made up of parts developed in all three tissues. The relations between membrane and cartilage bones have been fully discussed by Schleip (1904, p. 417).

In naming the individual bones of the mandible in modern fishes I have followed the suggestions of Goodrich (1930, p. 303), speaking of the bones usually called the splenial and articular or derm-articular as the pre-articular and angular, in order to facilitate comparison with the bones of other vertebrates. I have also used the term retro-articular for the so-called angular, following Boker (1913, p. 388).

THE MANDIBLE IN DIPNOI.

A longitudinal section through the mandibular articulation of a 38-cm. example of Protopterus ethiopicus, passing through the posterior end of the mandible and the lower end of the quadrate, is shown in Text-fig. 1. The same conventions are used as in my earlier paper on epiphyses in fishes, that is black for bone, white for cartilage, and an irregular network for connective tissue.

Meckel's cartilage (M.c.) is greatly expanded at its posterior end, forming a deep concavity for the articulation of the quadrate, while anteriorly it narrows down to a shaft which traverses the whole length of the mandible. The cartilage cells are of immense size, so that although the figure is drawn under very low magnification it is still possible to show the individual cells accurately. They are all rounded and evenly distributed through the cartilage matrix except those lying just beneath the perichondrium and those forming the articular surface,

which are more closely packed. There are no special growth zones differentiated in the cartilage, and there are no areas of calcification or ossification. The whole cartilage can therefore grow evenly throughout its extent, expanding in a way similar
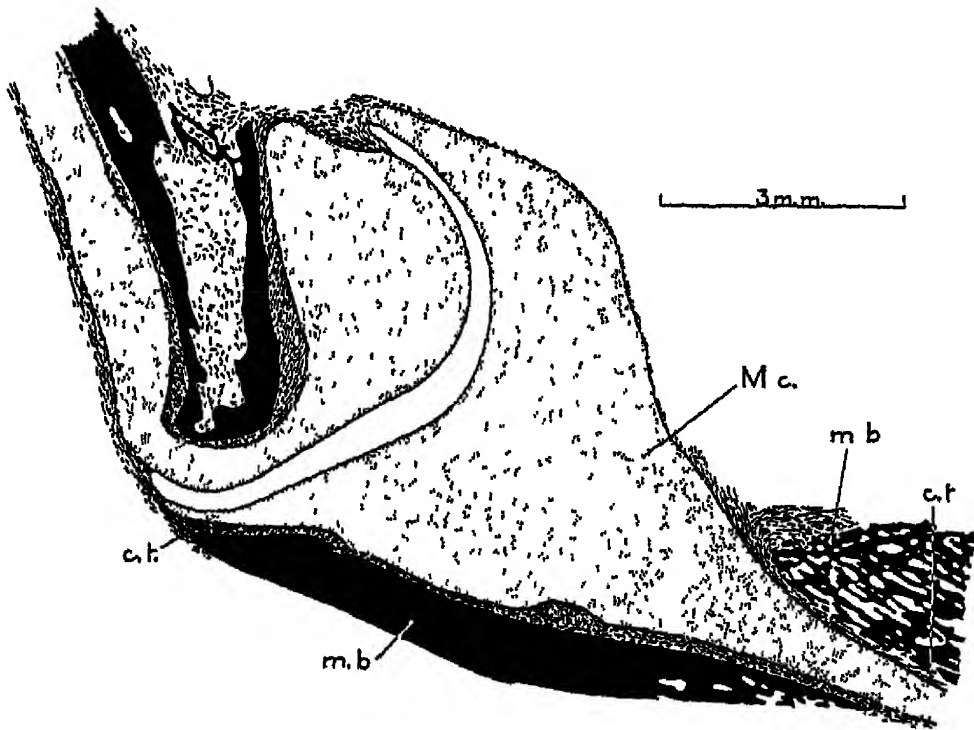
TEXT-FIG. 1.

Longitudinal section of the posterior end of the mandible and of the quadrate of Protopterus ethiopicus. *c t*, connective tissue; *m.b.*, membrane bone; *M.c.*, Meckel's cartilage.

to that seen in the cartilages of elasmobranchs and in the early embryos of all bony animals, but not usually found in their later stages.

Two membrane bones (*m.b.*), usually spoken of as the 'splenial' and 'angular', are seen in section near Meckel's cartilage, but separated from it in their whole extent by a layer of connective tissue (*c.t*) which forms a perichondrium for the cartilage and a periosteum for the bone. No perichondral or endochondral bone is formed, and the membrane bones, being surrounded on all sides by connective tissue, can expand in all directions by the addition of new bone to the surface of the old.

It is now known that the modern Dipnoi are not truly

primitive in their lack of endochondral bone, for Watson and
Gill (1923, p. 208) have shown that in the Devonian form
D i p t e r u s there was a well-developed endochondral articular
bone. But even though the Dipnoi have undergone secondary
simplification, the mode of growth of the mandible is probably
representative of a stage in the evolution of all bony animals,
the stage before the dermal bones invaded the membranous
tissues of the jaw and came into relation with Meckel's cartilage.

THE MANDIBLE OF POLYPTERUS.

The most primitive mandible available for study in living
bony animals is probably that of P o l y p t e r u s, for though it
shows certain specializations, for instance the well-developed
retro-articular process, the general structure seems to be fully
representative of that in the earliest bony fishes. It has been
carefully described by Allis (1922) and by Schmah (1934).

Meckel's cartilage is ossified by a large articular (*Art.*) which
completely subdivides the once continuous cartilage into three
separate parts, the main mass (*M.c*), a very small articular part
(*a.c.*), and a larger mass forming the cartilaginous part of the
retro-articular process (*c.r.a.p*). The main mass forms a rod
lying in the axis of the mandible, partly ensheathed by the
dentary and angular (*Ang.*). The posterior end of this cartilage
is firmly embedded in the articular, which is advancing into the
cartilage by ossification of the perichondrium (*p.p.Art.*), and
by replacement of the cartilage by bone marrow (*b m*) and
endochondral bone (*e.p.Art.*). Schmah (1934, p. 370) has
described a medio-Meckelian ossification, which is found near
the middle of the length of the cartilage, but my sections un-
fortunately do not reach so far forwards.

The articular part of Meckel's cartilage (*a c.*) is very small in
amount, forming the concavity for articulation with the quad-
rate. The actual joint-surface is covered by a thin layer of
fibro-cartilage (*f.c.a.*). Allis (1922, p. 247) on macroscopic
examination could detect no cartilage here, but my sections
leave no doubt as to its presence.

The retro-articular process consists of a backwardly pro-
jecting part of the articular, covered by a cap of hyaline cartilage

(*c.r.a.p.*). The bone shows endochondral and perichondral parts (*e.b.* and *p.b.*) related to the cartilage just as the corresponding parts of the anterior end of the articular are related to the main mass of the cartilage, and here again the bone enlarges by ossification of the perichondrium and replacement of the
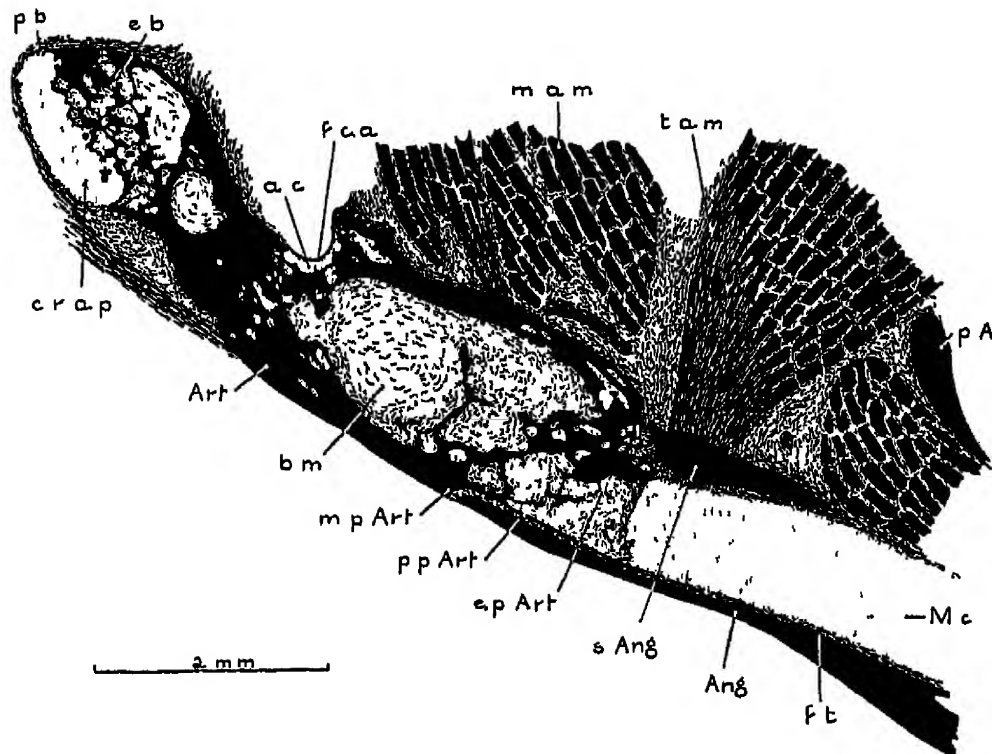
TEXT-FIG 2.

Longitudinal section of the posterior end of the mandible of P o l y -
p t e r u s   *a c.*, articular cartilage, *Ang.*, angular, *Art*, articular;
b.m., bone marrow; *c.r.a.p*, cartilaginous part of retro-articular
process; *e b.*, endochondral bone; *e p Art*, endochondral part of
articular; *f c a.*, articular fibro-cartilage; *f.t.*, fibrous tissue,
m a m., adductor mandibulae muscle; *M.c.*, Meckel's cartilage,
m p.Art., membranous part of articular; *p.A*, pre-articular,
p.b, perichondral bone, *p p.Art*, perichondral part of articular,
s.Ang., sesamoid part of angular; *t a m.*, tendon of adductor
mandibulae.

cartilage. The arrangement of the cartilage and bone is reminiscent of that in the epiphyses of the branchial bones of fishes (Haines, 1934), but a special growth zone of flattened cells is not developed here or in any other part of Meckel's cartilage.

The structure of the angular (*Ang.*) in P o l y p t e r u s is of the greatest interest, as it shows a stage in the development

of the sesamoid articular, a bone found in teleosts for which no
satisfactory explanation has hitherto been found. Inserted into
the dorsal surface of the mandible is the large adductor mandi-
bulae muscle (*m.a.m.*). This is divided into three parts, a pos-
terior inserted into the articular, a middle which gives rise to
a large tendon (*t.a.m.*) inserted into a specially developed part of
the angular (*s.Ang.*), and an anterior part inserted between the
pre-articular (*p.A.*) and Meckel's cartilage. A detailed examina-
tion shows that this part of the angular grows by the direct
ossification of the tendon itself, in a manner reminiscent of the
development of sesamoid bones In typical teleosts the sesamoid
articular develops in precisely this way, so that it appears that
this part of the angular in P o l y p t e r u s becomes split off in
other fishes as the sesamoid articular.

The dentary (not shown in the diagram) and the angular
are purely membranous in character, being separated every-
where from Meckel's cartilage by a layer of fibrous tissue (*f.t.*).
The articular, besides its perichondral (*p.p.Art.*) and endo-
chondral (*e.p.Art.*) parts, has also invaded the connective
tissues, so that its membranous part (*m.p.Art.*) lies in the same
plane as the angular, and helps to form the outer and ventral
surfaces of the mandible. This invasion of the connective
tissues is a specialization not found in the more primitive fishes.
The mode of growth will be discussed when the mandible of
E l o p s has been described.

The Growth of the Mandible in the Primitive Teleost, Elops saurus.

The cranial osteology of E l o p s s a u r u s, one of the most
primitive of the living Teleostei, has been carefully described
by Ridewood (1904). Two examples of this fish, known in South
Africa as the Eastern Province springer, 9 cm. and 21 cm. in
length, were available for study, and sections from their man-
dibles are shown in Text-figs. 3 and 5. The species reaches a
great size, and the larger of the two specimens is not more than
a third of its adult length. The same conventions are used here
as in Text-fig. 1 with the addition of stippling for calcified
cartilage. A wax and blotting-paper reconstruction of the

posterior end of the mandible of the smaller specimen is shown in Text-fig. 4.

In the smaller specimen, Text-figs. 3 and 4, Meckel's cartilage (*M.c.*) forms a continuous core running through the whole length of the mandible. It expands posteriorly to form an arti-
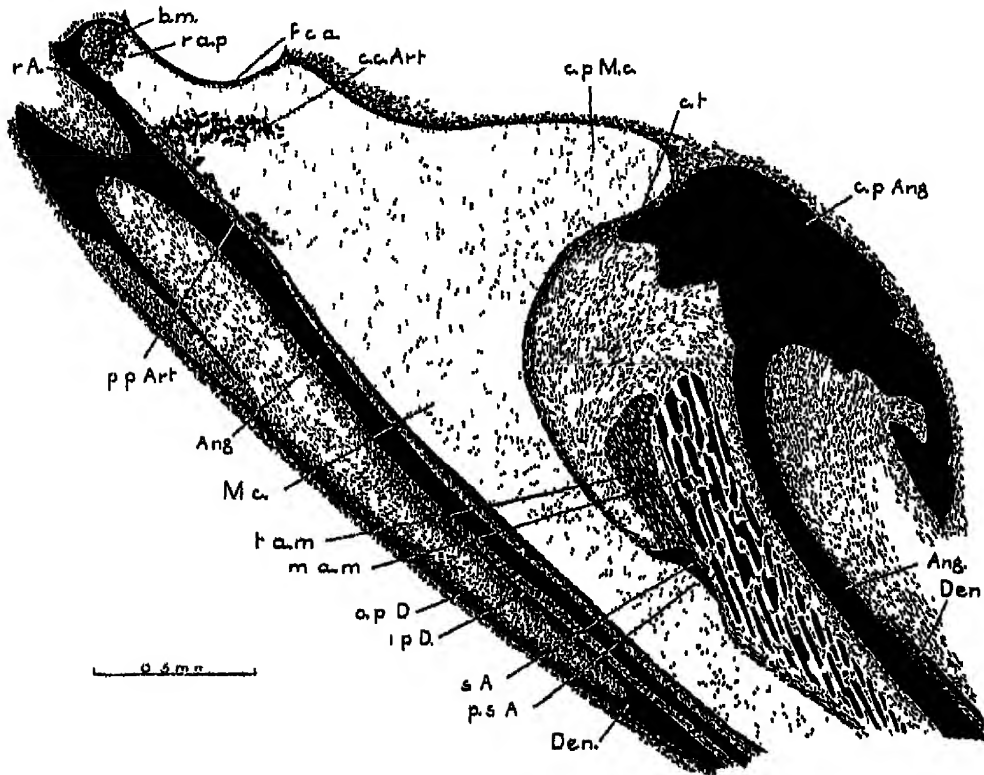
TEXT-FIG. 3.

Longitudinal section of the posterior end of the mandible of a 9-cm. Elops saurus. *Ang.*, angular; *b.m.*, bone marrow; *c.c.Art.*, calcified cartilage associated with articular; *c.p.Ang.*, coronoid process of angular; *c.p.M.c.*, coronoid process of Meckel's cartilage; *c.t.*, connective tissue; *Den.*, dentary, *f.c.a*, articular fibro-cartilage; *i.p.D.*, inner plate of dentary; *m.a.m.*, adductor mandibulae muscle; *M.c.*, Meckel's cartilage; *o.p.D.*, outer plate of dentary; *p.p.Art.*, perichondral part of articular; *p.s.A* , process for sesamoid articular; *r.A.*, retro-articular; *r.a p.*, retro-articular process; *s.A.*, sesamoid articular; *t.a m* , tendon of adductor mandibulae.

cular surface, covered by a layer of fibro-cartilage (*f c.a.*). Superiorly the posterior end of Meckel's cartilage is expanded into a large cartilaginous coronoid process (*c.p.M.c.*), similar to that found in Amia and Lepidosteus (Allis, 1897, and Parker, 1882), but not usually found in Teleostei. Postero-

inferiorly a small retro-articular process (*r.a.p.*) is present, but hardly projects beyond the articular surface. The shaft of Meckel's cartilage is fairly uniform in width, except where it forms a small projection which carries the sesamoid articular (*p.s.A.*).

Over the greater part of Meckel's cartilage the cells are rounded and evenly spaced, for there are no specialized growth zones of flattened cells such as are found in epiphyses. The cells of the shaft of the cartilage are rather larger than those of the expanded posterior end, and are often flattened in a direction at right angles to the long axis of the cartilage. Except in the process for the sesamoid articular the cells of the shaft are not hypertrophied or calcified, and it will be shown later that they are capable of further division.

The tip of the retro-articular process is covered by a cap of bone, the retro-articular (*r.A.*), which has been laid down in the perichondrium of the process. It is continuous anteriorly with the neighbouring membrane bone, the angular (*Ang.*), but this is a specialization peculiar to E l o p s , for as will be shown later it is separate in most bony fishes, including the more primitive forms, L e p i d o s t e u s and A m i a . Where it is surrounded by the angular, the cartilage of the retro-articular process is irregularly calcified, and a small part of the extremity has been eroded and replaced by bone marrow (*b.m.*), but no endochondral bone has yet been formed.

More anteriorly a thin plate of bone has been laid down on Meckel's cartilage by the perichondrium. This is the peri-chondral part of the articular (*p.p.Art*), a bone which is not found in the Teleostei outside the Clupeiformes. The endochondral part is not yet developed, but its position is marked out by an area of calcified cartilage (*c.c.Art.*) which extends inwards from the perichondral bone below the articular surface for the quadrate.

There are three dermal bones in the mandible of E l o p s , the angular (*Ang.*), the dentary (*Den.*), and the sesamoid articular (*s.A.*). The angular and dentary form a pair of imperfect cones which fit closely one into the other, the angular into the dentary (Text-fig. 4); while the sesamoid articular is

a small bone attached only to Meckel's cartilage. Inferiorly the angular consists of a single plate of bone, continuous behind with the retro-articular, and separated from Meckel's cartilage
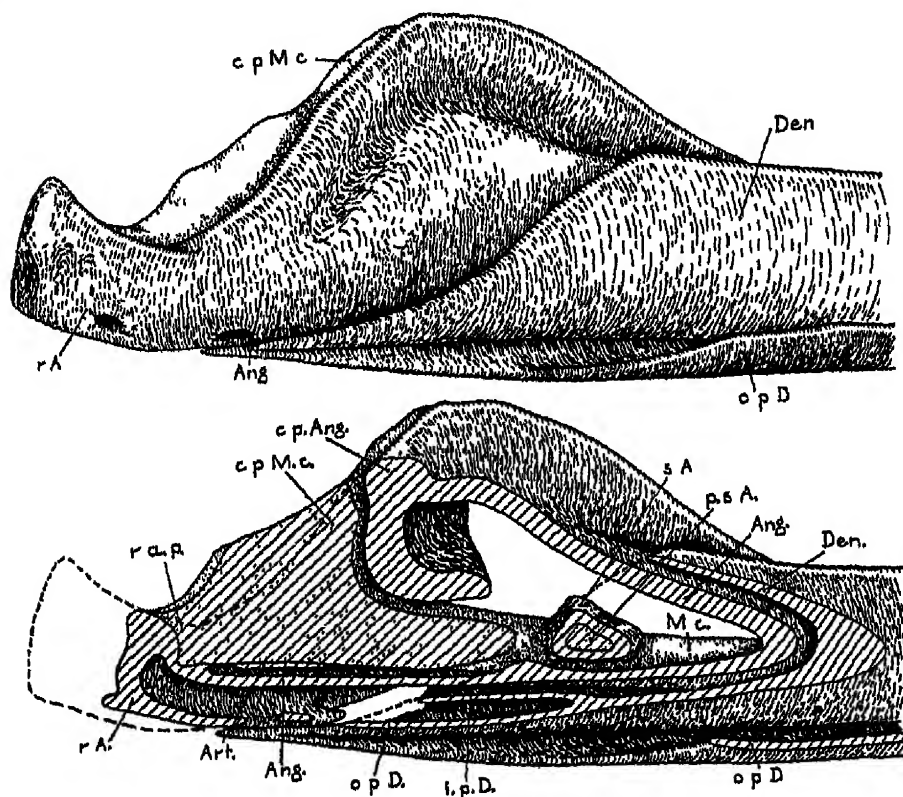
TEXT-FIG. 4.

Reconstruction of the posterior end of the mandible of a 9-cm. Elops saurus in lateral view *Ang.*, angular; *Art*, articular; *c.p.Ang.*, coronoid process of angular; *c.p.M.c*, coronoid process of Meckel's cartilage; *Den.*, dentary; *i.p D.*, inner plate of dentary; *M.c*, Meckel's cartilage; *o p.D*, outer plate of dentary; *p.s.A.*, process for sesamoid articular; *r A.*, retro-articular; *r.a.p*, retro-articular process; *s.A.*, sesamoid articular.

and the attached articular by a layer of connective tissue (Text-fig. 3, *c.t.*). Laterally it is less closely connected to the cartilage and the attached sesamoid articular. Superiorly the bone is separated from the cartilage by the mass of the adductor mandibulae muscle (*m.a.m.*) and its tendinous part which is inserted into the sesamoid articular (*t.a.m.*), and it forms an expanded coronoid process (*c.p.Ang.*) covering the antero-lateral surface of the cartilaginous coronoid process (*c.p.M.c.*), to which it is

attached by a layer of fibrous tissue. Medially neither Meckel's cartilage nor its coronoid process are covered by bone, so that in this direction the cartilage is free to expand during growth.

The posterior end of the dentary is formed of an inner plate (*i.p.D.*) which lies parallel to the outer surface of the angular
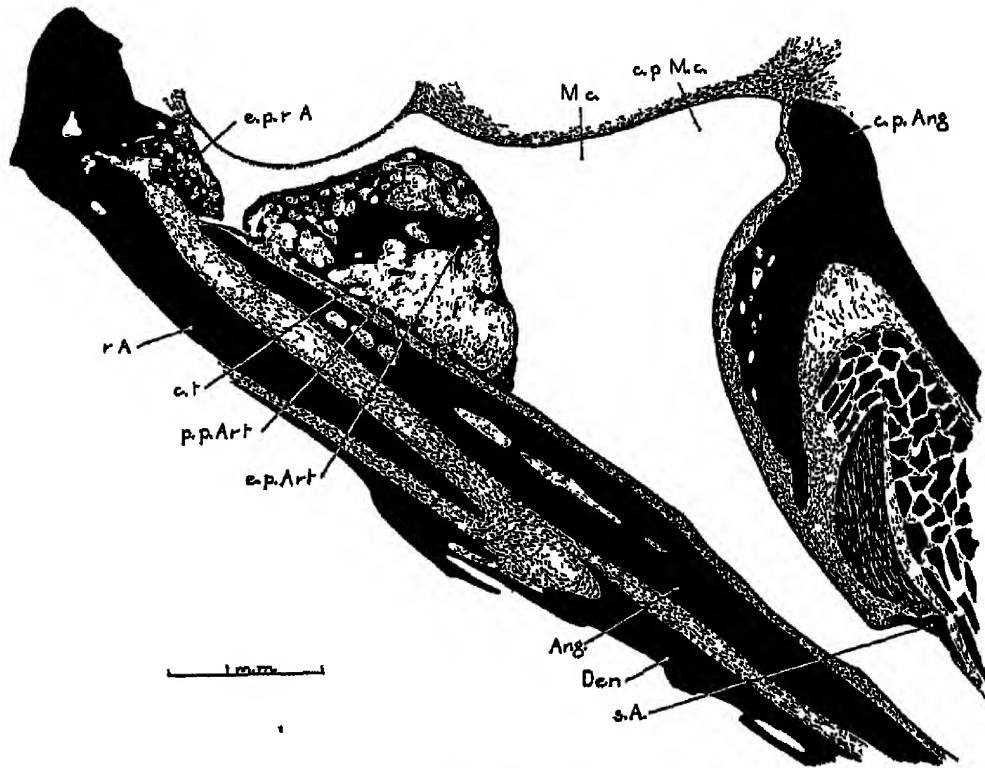
TEXT-FIG. 5.

Longitudinal section of the posterior end of the mandible of a 27-cm. Elops saurus. *Ang.*, angular; *c.p Ang.*, coronoid process of angular; *c p.M.c* , coronoid process of Meckel's cartilage, *c t* , connective tissue; *Den* , dentary; *e p.Art.*, endochondral part of articular; *e.p r A.*, endochondral part of retro-articular, *M.c.*, Meckel's cartilage; *p.p Art* , perichondral part of articular, *r A* , retro-articular; *s A* , sesamoid articular.

and is attached to it by dense fibrous tissue, and of outer plates (*o.p.D.*) which add to the width of the bone, and which overlap the anterior end of the retro-articular. The inner and outer plates are continuous at their margins.

The sesamoid articular (*s.A.*) is closely attached to the cartilage, which shows a thin layer of calcification underlying it. The bone is moulded over a small process of Meckel's cartilage (*p.s.A.*) so that it appears as a cap over the cartilage in Text-

fig. 3, and as a circle surrounding it on the cut surface of Text-fig. 4. It gives attachment to the tendon of the adductor mandibulae (*t.a.m.*).

A longitudinal section from the older specimen of E l o p s s a u r u s is shown in Text-fig. 5. The same conventions are used as in the earlier figures, but since the scale in this diagram is smaller than in Text-fig. 3, the cartilage cells are too small to draw, and Meckel's cartilage is left blank (*M.c.*). The general arrangement and proportions of the bones are little altered, and need not be described again. The perichondral part of the articular (*p.p.Art.*) has greatly increased in area by growing over the cartilage. It is perforated at several points and the perichondral, now the periosteal, tissues have invaded the cartilage as young bone marrow, and have laid down bone forming the endochondral part of the articular (*e.p.Art.*)

In Text-fig. 6 a small part of a section through the articular is shown under higher magnification. At the advancing margin of the endochondral bone the cartilage cells become hypertrophied, and around the cells the matrix is calcified. The calcification is irregular, so that a lighter zone (*l.z.*) and a darker zone (*d.z.*) are often seen. The marrow, which is left blank in this drawing, erodes the calcified cartilage, forming the usual bays of erosion (*b.e.*) seen in endochondral ossification (Haines, 1934). The bone is laid down on the remains of the calcified cartilage, which sometimes persists as a core in the trabeculae of bone (*c.t.b.*). Thus in this fish endochondral bone formation is an important mechanism in the growth of the mandible. In some more specialized teleosts to be described later endochondral bone is reduced or absent.

In the 9-cm. stage the cartilage at the tip of the retro-articular process had already been eroded by bone marrow beneath the perichondral part of the retro-articular. In the specimen shown in Text-fig. 5 the cartilage has been further eroded and endochondral bone has been laid down (*e.p.r.A.*), but the mass of this bone is not so great as that of the articular, though the process of erosion began earlier. The endochondral part anchors the retro-articular, and the angular, which in E l o p s is continuous with it, to the retro-articular process.

Apart from changes in size and the development of the endochondral parts of the retro-articular and articular there is little difference in the arrangement of the bones in the two specimens of Elops described. The question now arises how this harmony in the growth of the various parts of the bone
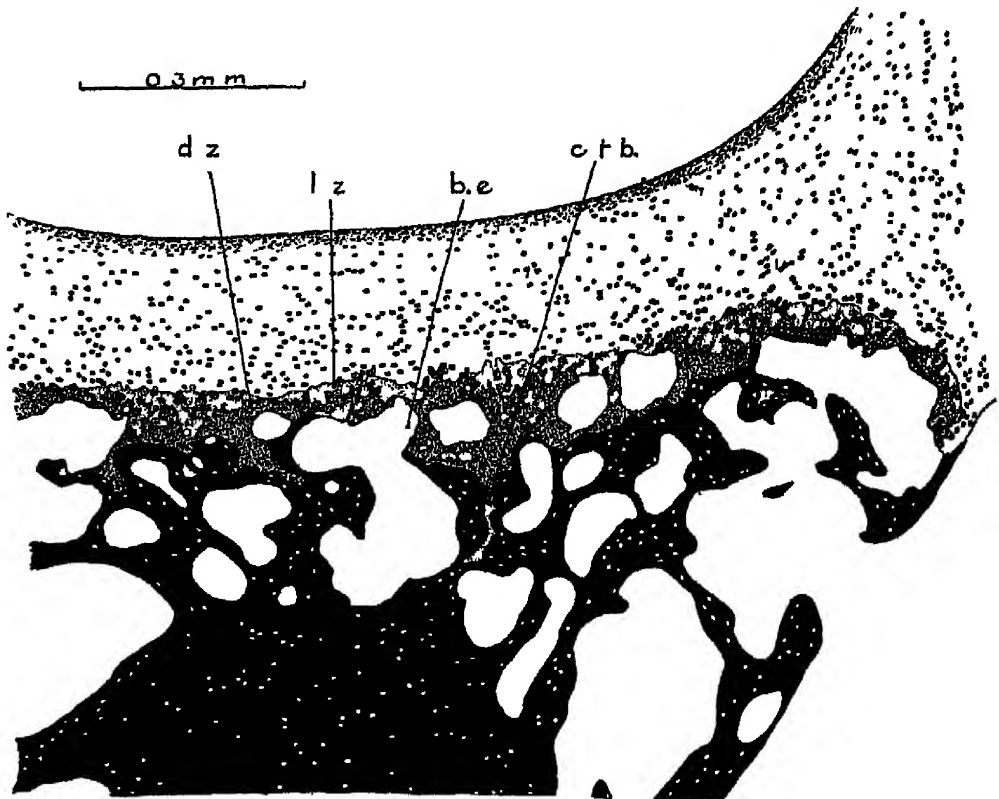
TEXT-FIG. 6.

Section from the articular part of the mandible of Elops saurus.
b.e., bay of erosion; c t.b., cartilage in bony trabecula; d.z., darker
zone; l.z, lighter zone.

is brought about. In Text-fig. 7 three stages in the growth of the bone are represented in diagrammatic form. The bone around the coronoid process is not represented. In the sections shown in Text-figs. 3 and 5 the posterior end of the angular appears to surround the cartilage, but the model shown in Text-fig. 4 shows clearly that the cartilage is free to expand on its medial surface.

There are three recognizable points along the length of the cartilage by which its growth can be determined, the positions

of the endochondral parts of the retro-articular (*e.p.r.A.*) and articular (*e.p.Art.*), and of the sesamoid articular (*s.A.*). The fact that these spread out evenly during their growth indicates that the cartilage is growing evenly throughout its length, and that there are no special zones where growth is intense while
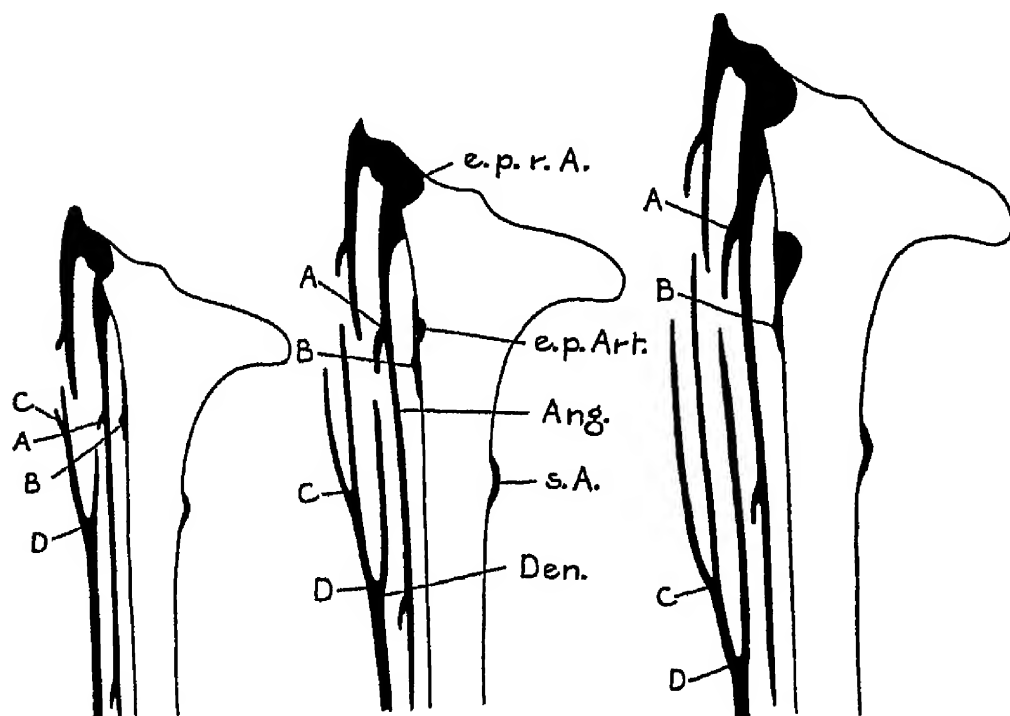
TEXT-FIG. 7.

Diagrams illustrating the growth of the mandible of Elops saurus. *Ang.*, angular; *Den.*, dentary; *e.p.Art*, endochondral part of articular; *e p r A.*, endochondral part of retro-articular; *s.A.*, sesamoid articular.

the rest of the cartilage is quiescent. This conclusion is confirmed by the absence of any zone of closely packed flattened cells such as are usually found in regions specialized for growth.

Thus the cartilage is a regularly growing structure which determines by its growth the position of the three bones attached to it. But connected to the endochondral part of the retro-articular (*e.p.r.A*) through the membranous part is the whole of the angular (*Ang.*), and this bone must therefore move with the retro-articular process. Thus the point A on the angular, originally opposite the point B on the articular, becomes in older specimens relatively displaced towards the articular end of the

jaw. The connective tissue layer which lies between the angular and the cartilage with the attached articular acts as a plastic bed which allows the dermal bone to slide over the cartilage and perichondral bone.

The dentary is pulled forward by the growth of the anterior end of Meckel's cartilage, just as the angular is pulled backward by the posterior end, so that the points o and A which lie opposite one another in the young bone are pulled apart as the fish grows. Similarly the point D, originally opposite the sesamoid articular (s.A.) is displaced anteriorly.

The connective tissue lying between the dentary and the angular allows the sliding of the one bone over the other. As the bones are forced apart by the growth of the cartilage, their overlap is maintained by the growth of new bone at their margins, and the functional rigidity of the mandible as a whole is maintained.

Further, since the structure of the mandible in Polypterus is similar to that in Elops, the mechanism of growth is presumably the same. In Polypterus as in Elops a layer of fibrous tissue separates the cartilage from the membrane bones, and though the relatively larger size of the articular must involve a compensating increase in the rate of growth of the unossified cartilage to make up for the absence of growth in the ossified part, no special growth zones are developed in the cartilage.

I have had no opportunity to examine the mandibles of Amia and Lepidosteus, but there is no reason to believe that any essential difference would be found in their structure. It appears probable that one type of growth has persisted among the more primitive fishes including some teleosts, while the more specialized teleosts now to be described have developed an entirely new mechanism.

The Growth of the Mandible in the Specialized Teleosts, Tetrodon and Notopogon.

As a contrast to the arrangement in Elops part of the mandible of a highly specialized teleost, a young specimen of the blaasop, Tetrodon honkenii, is shown in Text-fig. 8.

The simple uneroded form of the cartilage in this fish is probably derived secondarily from more complex forms, but these will be better understood if T e t r o d o n is described first, for in it the growth zones of the cartilage are shown with exceptional clarity.

At the articulation the cartilage forms a flattened cone which lies in a shallow depression of the articular bone. This articular cartilage is divided into two parts, a layer of fibro-cartilage (*f.c.a.*) forming the actual joint surface, and a mass of calcified cartilage (*c.c.a.*) lying more deeply. Through narrow prolongations of this cartilage the articular cartilage becomes continuous with two large masses of hyaline cartilage, the main mass of Meckel's cartilage (*M.c.*) anteriorly, forming the main axis of the mandible, and its retro-articular process (*r.a.p.*) at the angle of the jaw.

The single angular bone appears in the section in three parts (A, B, and C), but these are parts of a single structure, the bone being continuous around the narrow necks of calcified cartilage which connect together the articular cartilage, the retro-articular process, and the main mass of Meckel's cartilage. The structure of the bone is light and spongy as described by Stephan (1900, p. 387) in the P l e c t o g n a t h i. It is chiefly developed in the membranous tissue of the jaw (*m.p.Ang.*), but ossification has spread into the perichondrium (*p.p.Ang.*) where it forms a thin continuous casing for Meckel's cartilage.

The retro-articular, quite separate from the angular, closely surrounds the terminal part of the retro-articular process, which shows a thin area of calcification (*c.c.r.A.*) immediately deep to the bone. This bone again is developed partly in the perichondrium of the retro-articular process (*p.p.r.A.*), and partly in the membranous tissue surrounding it (*m.p.r.A.*).

The dentary (*Den.*), only a part of which is shown in the figure, lies outside the angular, its most posterior part reaching the retro-articular. A thin perichondral part (*p.p.D.*) surrounds the anterior part of Meckel's cartilage just as the perichondral part of the angular surrounds its posterior part. Meckel's cartilage between these two bones is surrounded by unossified perichondrium.

The sesamoid articular (*s.A.*) is a conical bone widely separated from Meckel's cartilage by a thick mass of connective tissue and by the anterior end of the perichondral part of the angular (*p.p.Ang.*). It is quite clear from this specimen that
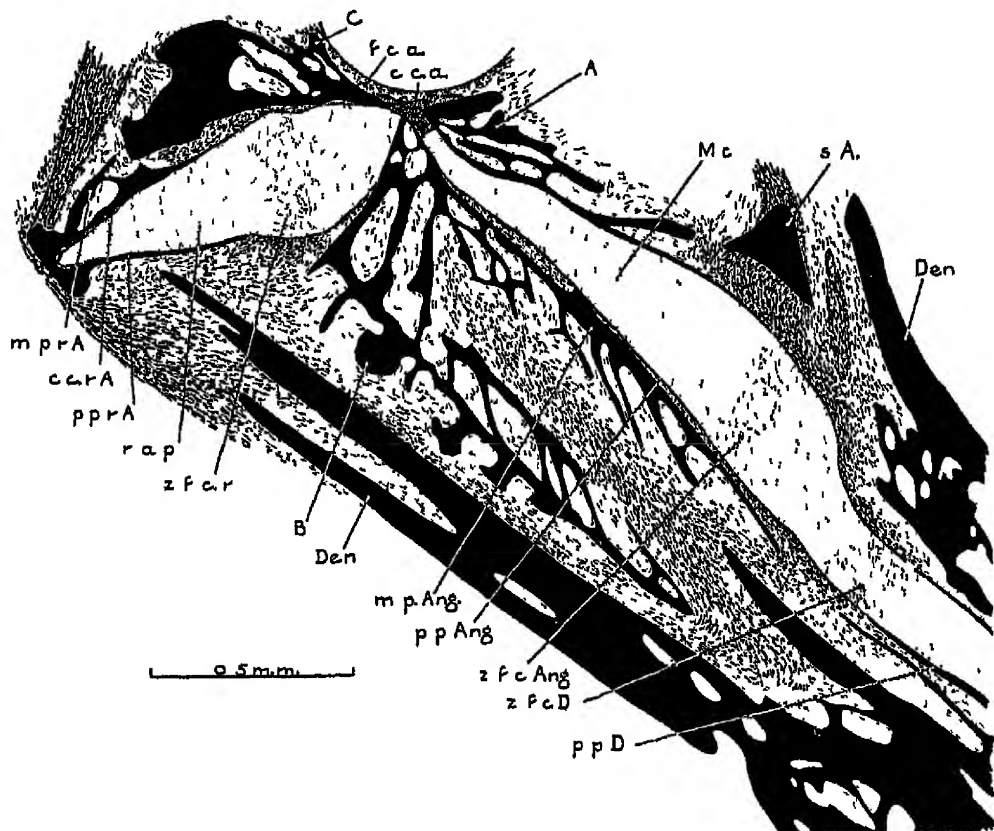
TEXT-FIG. 8.

Longitudinal section of the posterior end of the mandible of a 5-cm Tetrodon honkenii. *c.c.a.*, calcified articular cartilage; *c.c r A* , calcified cartilage related to retro-articular; *Den.*, dentary, *f c a.*, articular fibro-cartilage; *M c* , Meckel's cartilage; *m p.Ang.*, membranous part of angular; *m p r A* , membranous part of retro-articular, *p p Ang* , perichondral part of angular, *p.p.r.A.*, perichondral part of retro-articular, *p.p.D* , perichondral part of dentary; *r.a p.*, retro-articular process, *s A* , sesamoid articular; *z f c.Ang.*, zone of flattened cells associated with angular; *z f.c D* , zone of flattened cells associated with dentary; *z.f c.r* , zone of flattened cells of retro-articular process

it is a membrane bone, and that its close attachment to Meckel's cartilage in E l o p s is secondary.

Most of the cartilage shows large rounded cells, widely scat-

tered in the matrix, a condition found in tetrapods and in the branchial bones of fishes (Haines, 1934) to indicate inactivity of growth. But in three zones the cells are small, closely packed, and flattened so that they lie transversely to the longitudinal axis of the cartilage, a condition indicating intense growth activity in the direction of the axis. Two of these growth zones of flattened cells lie close together at the adjacent margins of the angular and dentary (*z.f.c.Ang.* and *z.f.c.D.*). The third lies in the retro-articular process (*z.f.c.r.*), and bears a similar relationship to the border of the retro-articular.

By the growth of the cartilage between the angular and the dentary these two bones are pressed apart. If the margins of the bones did not grow towards one another at the same time there would be an ever widening gap between them, but by growth of their various borders the right degree of overlap of their membranous parts and of approximation of their perichondral parts is maintained. Similarly the growth of the cartilage in the retro-articular process lifts the retro-articular away from the angular, but at the same time the retro-articular grows back over the cartilage towards the angular.

The structure of the jaw here described is quite different from that given by Stephan (1900, p. 359) with coloured figures of a 'tête articulaire du maxillaire inférieur de Tetrodon reticulatus'. His figures show typical endochondral bone formation, similar to that described here in Elops saurus.

The condition of the bone in Tetrodon is the outcome of a long series of specializations to be discussed later. Similar changes have led in the equally specialized but entirely unrelated Gasterosteiform fish, Notopogon macrosolen, to very similar results in the cartilage. But here the bone is massive and compact instead of being light and spongy, and the beautifully developed series of growth lines in the bone itself can be used to confirm and extend the conclusions reached from a study of the cartilage in Tetrodon.

In a 15½-cm. specimen of Notopogon, Text-fig. 9, the general shape of the cartilage with its expanded articular cone, its constricted neck, and its gradually widening central mass is very similar to that in Tetrodon. Below a typical fibro-

cartilage (*f.c.a.*) lies a thin layer of cartilage with very closely packed rounded nuclei passing into a thick mass of hyaline cartilage (*h.c.a.*) in which the cells are elongated and arranged in columns radiating from the constricted neck towards the articular surface. At the neck and for some distance down the
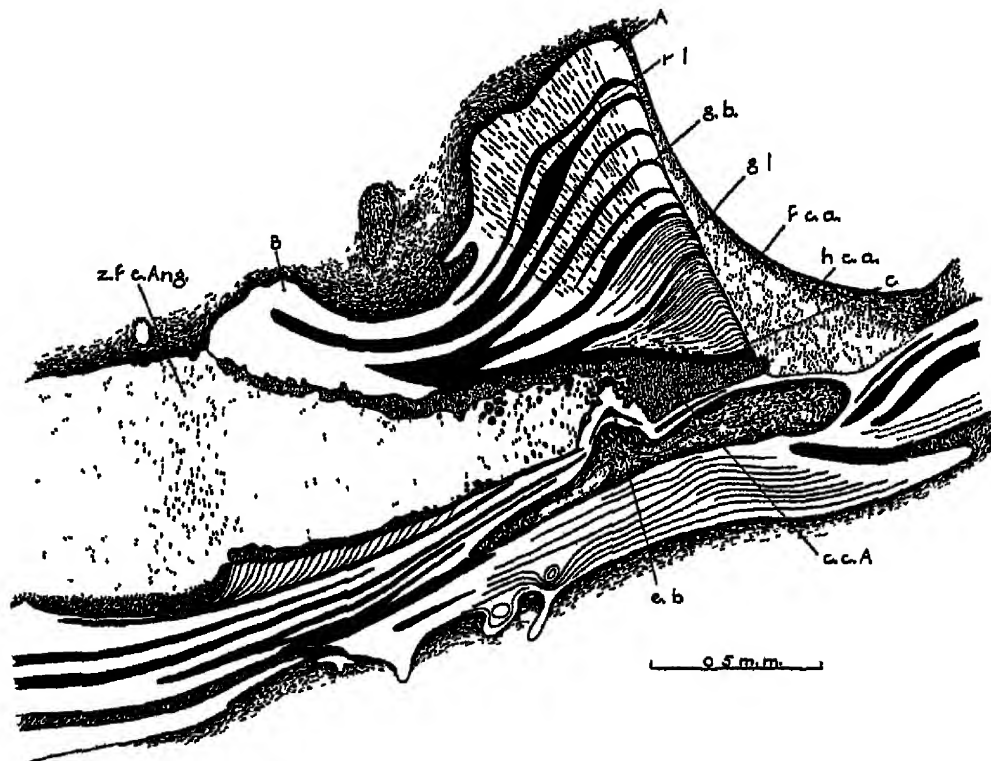
TEXT-FIG. 9.

Longitudinal section of the posterior end of the mandible of N o t o - p o g o n   m a c r o s o l e n.   *c c.A.*, calcified cartilage associated with angular; *e b.*, endochondral bone; *f.c.a.*, articular fibro-cartilage; *g.b.*, growth band, *g.l.*, growth line, *h c.a.*, hyaline articular cartilage; *r.l*, radial line; *z.f.c Ang*, zone of flattened cells associated with angular.

shaft the cartilage is calcified where it is surrounded by the angular bone (*c.c.A.*). A growth zone of flattened cells is developed in a position similar to that described in T e t r o d o n (*z.f.c.Ang.*).

In the bone itself two sets of markings are developed at right angles to one another. One set, the true growth lines and bands (*g.l.* and *g.b.*), form a series of curves lying parallel to one another and to the surface of the bone. The other series, the radial lines

(*r.l.*), are less conspicuous and only developed towards the periphery. The oldest growth lines are those lying nearest the cartilage, the youngest those at the periphery. It will be noticed that if any growth line be taken as representing the outline of the angular at a particular phase of its growth, say that labelled *g.l.*, the new bone belonging to the next lamella to be formed is laid down on its outer surface but not on its articular surface. Thus whereas the outer surface of the angular between the points marked A and B is formed entirely by the outer surface of the latest lamella to be laid down, the articular surface A to C is formed of the articular ends of the successive lamellae from the youngest to the oldest. Once formed this surface of bone which carries the articular cartilage has no further power of growth. The longitudinal growth of the bone must therefore depend entirely on growth at the opposite anterior end, and this conclusion is confirmed by the presence of a growth zone of flattened cells in the underlying cartilage. The articular cone of cartilage can grow in depth as the bony hollow it occupies deepens, but it has no power to lay down new bone on the surface of the old.

The growth of the mandible in T e t r o d o n or N o t o p o g o n differs from that in P r o t o p t e r u s or E l o p s chiefly in the sharp localization of the zones of active growth in the cartilage. In place of an even expansion of Meckel's cartilage distributed over its whole length, longitudinal growth is concentrated in two narrow specialized zones, and a third zone governs the growth of the retro-articular process. In each case these zones lie centrally to the bones whose displacement they cause, and the method of growth may be called the central mechanism. It will be shown later that this mechanism is widespread in teleosts. But in the branchial bones of all Teleostei examined another method of growth, the epiphysial mechanism, is found (Haines, 1934), and this is also found in the branchial bones of T e t r o d o n and N o t o p o g o n. So in these fish two mechanisms of bone growth, epiphysial and central, are found together, and it is useful to compare them as in the diagrams drawn in Text-fig. 10.

The two mechanisms are not entirely dissimilar In both the longitudinal growth depends on the zones of rapidly dividing

flattened cartilage cells (*z.f.c.*), but in epiphysial growth these lie near the ends of the cartilage, while in central growth they
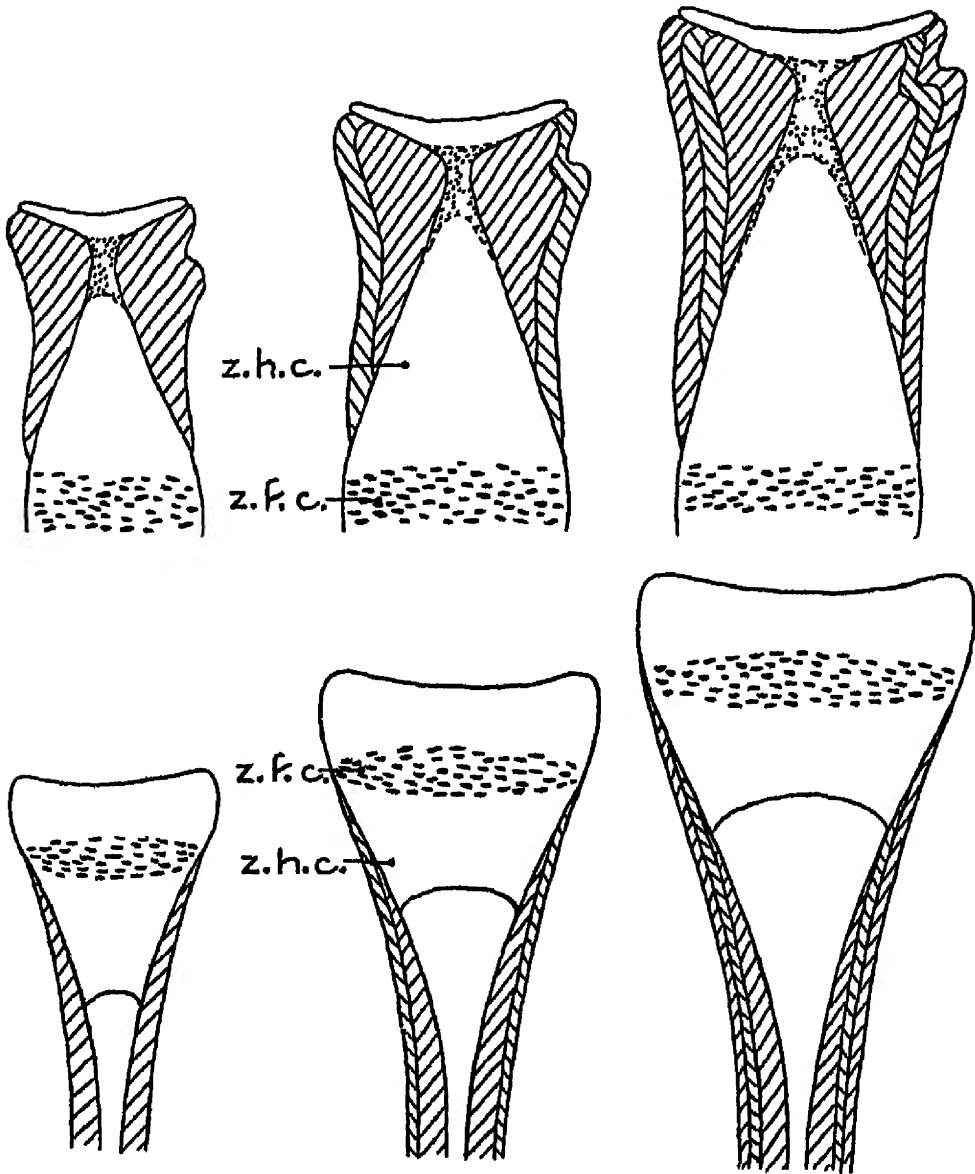
TEXT-FIG. 10.

Diagrams illustrating growth in an epiphysis (below) and growth by the central mechanism (above). *z f c*, zone of flattened cells; *z.h.c*, zone of hypertrophied cells.

lie close together near the centre of the cartilage. In both the cells derived from the growth zone become hypertrophied and the matrix enlarged (*z.h.c.*); but in epiphysial growth the single inert mass of cartilage so formed lies between the two growth

cartilages, while in central growth two masses are formed lying on either side of the growth cartilages. In epiphysial growth the central part of the mass of hypertrophied cartilage is usually eroded by marrow and replaced by endochondral bone. In T e t r o d o n the hypertrophied part of Meckel's cartilage is not eroded, but in the specimen of N o t o p o g o n figured a small area of erosion lined by endochondral bone (*e.b.*, Text-fig 9) has been formed, and in the fishes to be described later complete interruption of the continuity of the cartilage is the rule.

In both central and epiphysial growth the bone grows at its margins over the inert cartilage as fast as this is formed from the growing cartilage. In epiphysial growth there is but one bone forming the shaft and this grows at both its ends, while in central growth there are two bones developed in the perichondrium and these grow at their contiguous margins rather than at their articular ends.

How important the central mechanism may be in the economy of fishes is difficult to say. The only certain statement I can make is that wherever central growth was found in the mandible the quadrate region also grew by this mechanism.

THE MANDIBLE IN THE MORE TYPICAL TELEOSTS, SARDINA, MUGIL, AND TRIGLA.

The Cape sardine, S a r d i n a s a g a x, is a good type for the study of the growth of Meckel's cartilage. Two sections are shown in Text-figs. 11 and 12, one from a 3·5 and the other from a 29-cm. specimen.

In the younger fish Meckel's cartilage is a simple rod of uniform width, which forms the articular surface, and projects posteriorly as a conical retro-articular process. Around it lies the angular, cut in three segments (A, B, and C), which, when followed through other sections, are seen to be continuous with one another. A small sesamoid articular (*s.A.*) receives the insertion of the tendon of the adductor mandibulae muscle (*t.a.m.*). The retro-articular process is covered by a cap of perichondral bone, the perichondral part of the retro-articular

(*p.p.r.A.*), while the membranous part (*m.p.r.A.*) extends into the connective tissues of the jaw.

The cartilage, though still very simple in shape, already shows considerable differentiation in its structure. In the main parts of the shaft most of the cells are somewhat flattened at right angles to the longitudinal axis of the cartilage (*f.c.*), but near the perichondrium they are rounded (*r.c.*), or even elongated
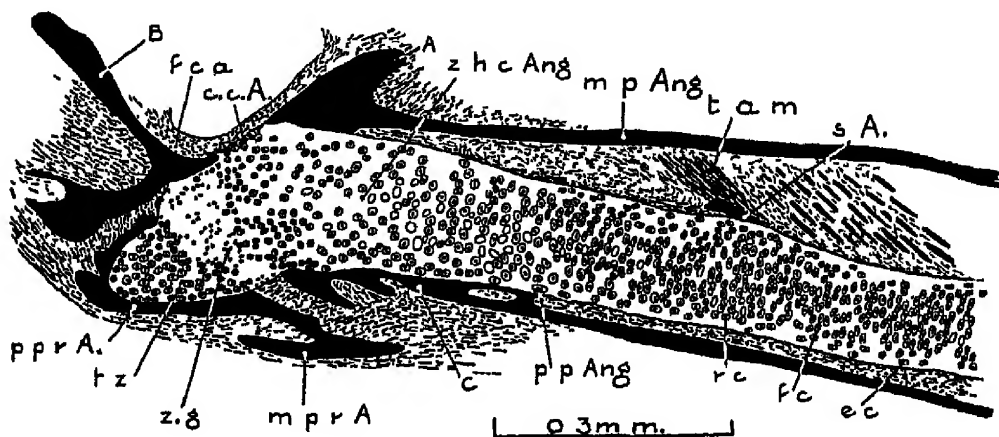
TEXT-FIG 11

Longitudinal section from the posterior end of the mandible of a
3·5 cm. Sardina sagax   *c c A.*, calcified cartilage associated
with angular, *e.c* , elongated cell; *f.c.*, flattened cell; *f.c a* , articular
fibro-cartilage; *m p Ang* , membranous part of angular; *m.p.r A.*,
membranous part of retro-articular, *p p.Ang* , perichondral part
of angular; *p p.r.A* , perichondral part of retro-articular; *r c* ,
round cell; *s A* , sesamoid articular; *t a m.*, tendon of adductor
mandibulae; *t.z* , terminal zone, *z.h c Ang* , zone of hypertrophied
cartilage associated with angular; *z.g* , zone of growth.

in a direction parallel to the surface (*e c.*). In this region the perichondrium is completely unossified except where the sesamoid articular is invading it, and the membranous part of the angular (*m.p.Ang*) is separated from the cartilage by a thick layer of fibrous tissue. At this time therefore the cartilage is free to expand evenly in both transverse and longitudinal directions, just as is the cartilage of the shaft in the adult Elops, and the cells are correspondingly undifferentiated.

Nearer the articular surface the cells are rounder and more scattered (*z.h.c.Ang.*). Here the angular has invaded the perichondrium (*p.p.Ang*), and, as is shown by a study of the

adjoining sections, has grown round the cartilage so as to form a complete bony ring around it. This then is a zone where no further growth of the cartilage can occur, and the scattered appearance of the cells indicates the presence of a zone of hypertrophy. This zone lies both anteriorly to the articular
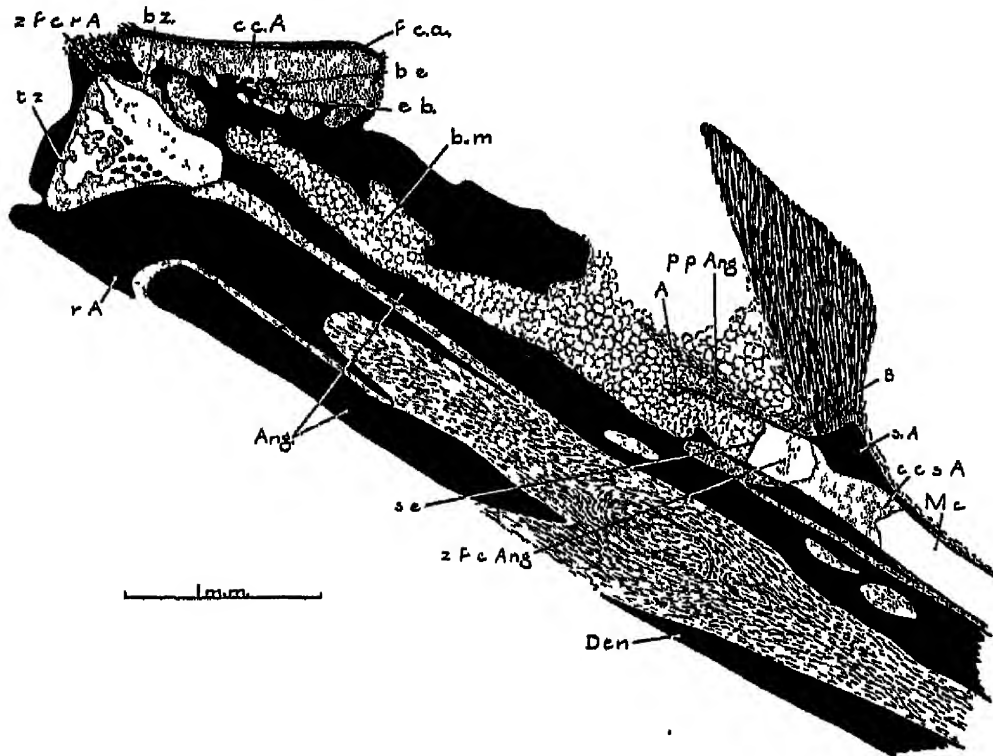
TEXT-FIG. 12.

Longitudinal section from the posterior end of the mandible of a 20-cm. Sardina sagax. *Ang.*, angular, *b.e.*, bay of erosion; *b.m.*, bone marrow; *b.z.*, basal zone; *c.c.A*, calcified cartilage associated with angular; *c.c.s.A*, calcified cartilage associated with sesamoid articular; *Den.*, dentary; *e.b.*, endochondral bone; *f.c.a*, articular fibro-cartilage; *M.c*, Meckel's cartilage; *p p.Ang.*, perichondral part of angular; *r.A.*, retro-articular; *s.A.*, sesamoid articular; *s.e.*, surface of erosion; *t z.*, terminal zone; *z.f.c.Ang.*, zone of flattened cells associated with angular; *z.f.c.r.A.*, zone of flattened cells associated with retro-articular.

surface (between A and c), and posteriorly to the surface (between B and c). Just below the fibro-cartilage forming the joint surface itself (*f.c.a.*) there is a small area where the cartilage is calcified (*c.c.A.*). Now in the present specimen the width of the cartilage rod where it is surrounded by bone is equal to that of the rest of the shaft, but since no further growth is possible,

this cartilage if it persisted in later stages would come to form narrow necks connecting the articular part with the retro-articular process and with the shaft. This condition is actually realized in Tetrodon, but in Sardina one of the necks, that to the shaft, is completely destroyed.

The terminal part of the retro-articular process (*t.z.*) is also incapable of further growth, since it is surrounded by the peri-chondral part of the retro-articular (*p.p.r.A.*). Thus the further expansion of the process depends on the cartilage which lies between the area surrounded by the angular and that surrounded by the retro-articular, and the cells are clearly differentiated by their small size and close spacing (*z.g.*). Later they become flattened as in the more typical zones of growth.

In the 29-cm. sardine (Text-fig. 12) destruction of the region which joins the articular part to the shaft has subdivided the cartilage into two portions, an articular part with the attached retro-articular process, and the shaft itself (*M.c.*). The shaft is still a rod of fairly even diameter, but though it has grown absolutely in size, it has decreased in importance relatively to the now massive membranous ossification of the angular (*Ang.*) and dentary (*Den.*). This narrow rod-like form is characteristic of most Teleostei. The shaft is hyaline in structure except where it is surrounded by the sesamoid articular (*s.A.*), which has constricted it and caused its calcification (*c c.s.A.*). This extension of the sesamoid articular around the cartilage is a specialization I have seen only in Sardina, but in several other fish its relationship to the cartilage is so close that it has been described as a cartilage bone (Schleip, 1904, and Boker, 1913, in Salmo, and Uhlmann, 1921, in Cyclopterus).

The posterior end of the cartilage of the shaft is embedded in the perichondral part of the angular (*p.p.Ang.*) which is advancing by ossification of the perichondrium (at B). A well-marked growth zone of flattened cells (*z.f.c.Ang.*) is shown diagrammatically in the cartilage between the angular and the sesamoid articular, a zone which serves to press apart these two bones. The surface at which the cartilage is undergoing erosion (*s.e.*) is smooth, so that no bays of erosion are formed and no endochondral bone is laid down. Soon after its formation

the perichondral bone itself is destroyed (at A) and replaced by fatty bone marrow (*b.m.*). Thus in S a r d i n a the cartilage near the articular area is destroyed and replaced by bone and bone marrow as in P o l y p t e r u s, but whereas in P o l y p t e r u s the replacing bone is the articular, in S a r d i n a it is the angular, a membrane bone. Further the whole mass of perichondral and endochondral bone is much reduced in comparison with the membranous ossifications.

The articular surface is made of the usual layer of fibro-cartilage (*f.c.a.*) overlying a large mass of calcified fibro-cartilage (*c.c.A.*), which grows by the addition of new tissue from the fibro-cartilaginous layer. On its deep surface this calcified mass is eroded by the bone marrow of the angular, with the formation of bays of erosion (*b.e.*) and of endochondral bone (*e.b.*).

Through a narrow neck the calcified cartilage becomes continuous with the retro-articular process. This is a biconical mass, consisting of a low cone of calcified cartilage embedded in the angular, forming a basal zone (*b.z.*), and a taller cone embedded in the retro-articular, forming a partly calcified terminal zone (*t.z.*), with an intermediate mass of hyaline cartilage containing a typical growth zone of flattened cells (*z.f.c.r.A.*).

Compared with P o l y p t e r u s and E l o p s, S a r d i n a shows full specialization of its cartilage into inert masses and special growth zones, and complete replacement of the articular by the angular. The retro-articular has increased in size by an enlargement of its membranous parts, while its endochondral part has been lost, so that the retro-articular process is an unbroken mass of cartilage. It seems probable that the condition of the cartilage in T e t r o d o n and N o t o p o g o n (as also in my specimens of H i p p o c a m p u s and F i s t u l a r i a), where it is uneroded by the angular, is secondarily derived from a more primitive, S a r d i n a -like, state.

A section through the mandible of another teleost, M u g i l c e p h a l u s, is shown in Text-fig. 18, and a more detailed view of the posterior part in Text-fig. 14. The general arrangement of cartilages and bones is similar to that in S a r d i n a, for here again the relatively narrow shaft of Meckel's cartilage is separ-

ated by an eroded space from the conjoint articular and retro-articular cartilage. The sesamoid articular is large, but normal in shape and position, being developed entirely in membrane, not partly in perichondrium as in Sardina. The articular cartilage is hyaline except for its fibro-cartilaginous articular surface (*f.c a.*) and its deeper calcified part (*c.c.A.*) which is
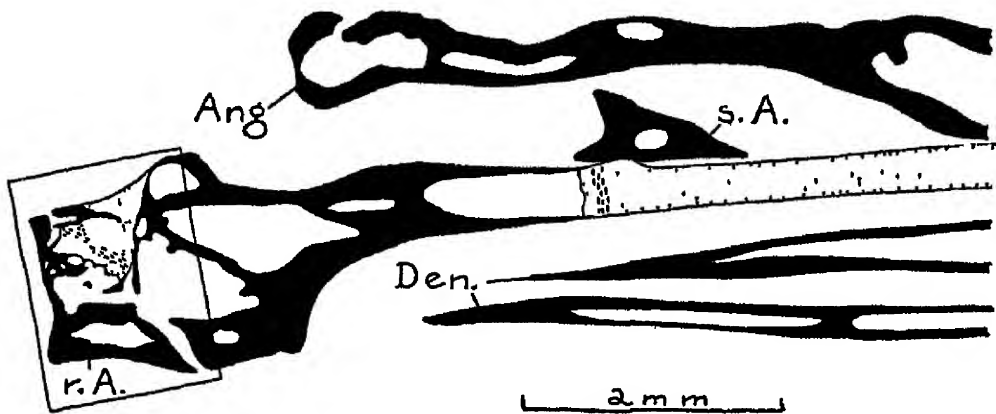
TEXT-FIG 13.

Low-power view of a longitudinal section of the posterior end of the mandible of M u g i l c e p h a l u s . *Ang* , angular, *Den* , dentary, *r.A* , retro-articular; *s A.*, sesamoid articular. The part in the rectangle is shown in Text-fig. 14.

continuous with the retro-articular process. This process is of particular interest, as it still shows in Mugil a large endo-chondral ossification, a part of the retro-articular which has been lost in Sardina and in most teleosts. I have also noted this ossification in Barbus anoplus.

The process shows three main zones, a basal zone (*b.z.*), a zone of flattened cells (*z.f.c.*), and a terminal calcified zone (*t.z.*). The basal zone is irregularly calcified, and the cells where recognizable are large and rounded. It rests in a conical depression of the angular bone similar to that which contains the articular cartilage. The zone of flattened cells is a typical region of growth, covered at its periphery by connective tissue (*c.t.*) which allows its expansion.

The terminal part of the retro-articular process is completely calcified. On the surface next to the zone of flattened cells an irregular line of calcification is seen advancing to surround the

hypertrophied cells as these are derived from the flattened cells. At the opposite surface the cartilage is eroded by the bone
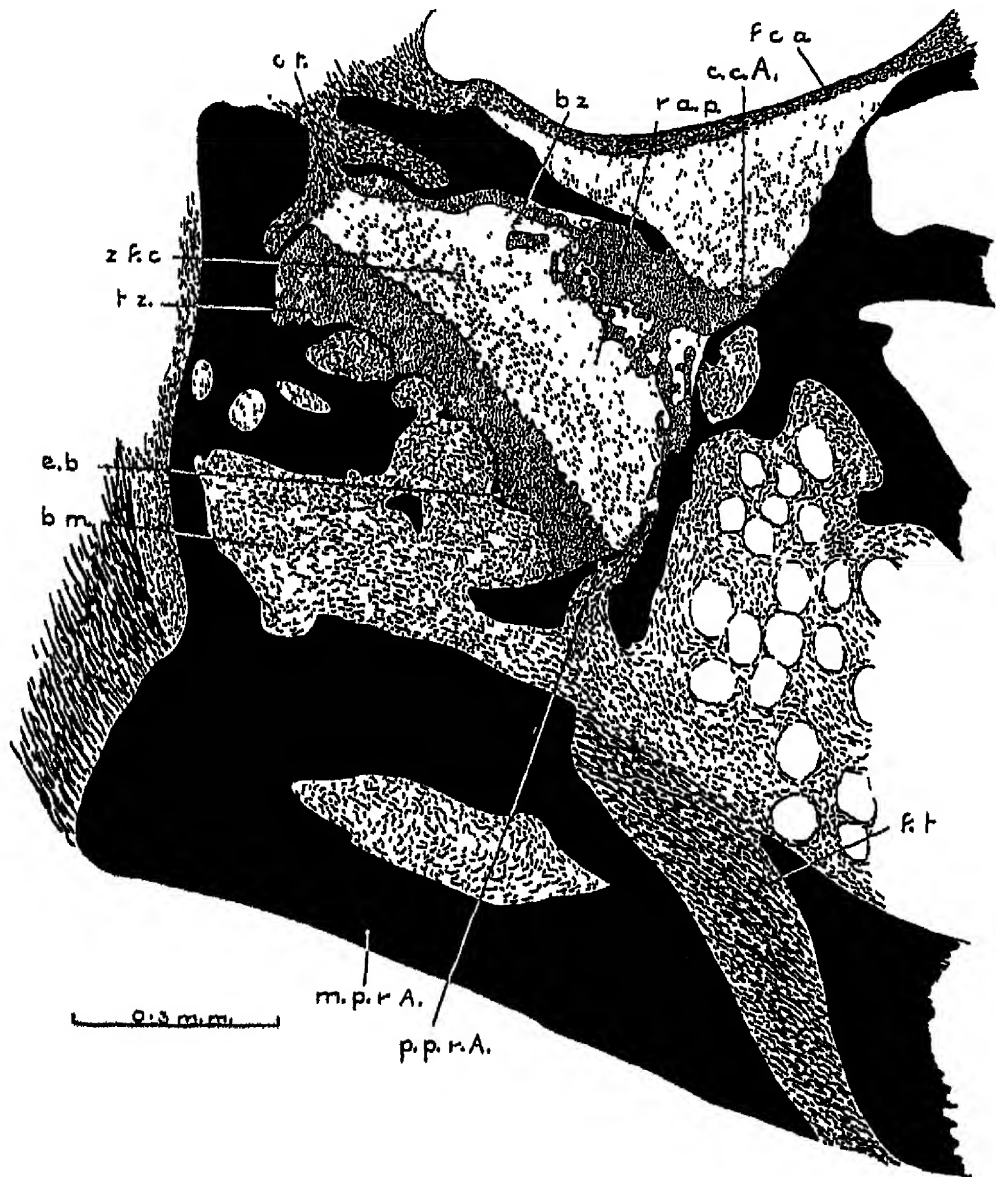
TEXT-FIG. 14.

High-power view of the part of the mandible of Mugil cephalus shown in the rectangle in Text-fig. 13. *b.z.*, basal zone; *b.m.*, bone marrow, *c.c.A.*, calcified cartilage associated with angular; *c.t.*, connective tissue; *e.b.*, endochondral bone; *f.c.a*, articular fibro-cartilage; *f.t.*, fibrous tissue; *m.p r A.*, membranous part of retro-articular, *p.p.r.A.*, perichondral part of retro-articular; *r a p*, retro-articular process; *z.f.c.*, zone of flattened cells; *t.z.*, terminal zone

marrow (*b.m.*), and on the irregular surface so cut out endo-chondral bone (*e.b.*) is laid down. The terminal zone is covered

by the retro-articular, whose perichondral part (*p.p.r.A.*) keeps pace with the growth of the cartilage. Continuous with the perichondral part of the retro-articular is a massive membranous part (*m.p.r.A.*), which forms the postero-inferior angle of the mandible. Anteriorly it is firmly attached to the angular by a layer of dense fibrous tissue.

Another teleost, T r i g l a c a p e n s i s, the Cape gurnard, has been chosen for description as it offers excellent illustrations of the processes described by Stephan (1900) as 'ossification mixte', and of the degree of complexity to which endochondral bone may be developed in fishes. A general low-power view of a section from the mandible is shown in Text-fig. 15. The section has been chosen to show as much as possible, but does not pass through the connexion between the shaft of Meckel's cartilage and the articular part, or through the retro-articular process. The shaft of the cartilage has two parts, an anterior (*M.c a.*) which is continued forwards to the dentary, and a posterior (*M.c.p.*) which in another section becomes continuous with the articular cartilage. The cartilage which lay between these two parts has been eroded and replaced by bone-marrow (*b.m.*), with a little endochondral bone (*e.b.*). In S a r d i n a and M u g i l the posterior part is completely destroyed.

The angular is spongy in texture. The anterior end of its perichondral part (*p.p.Ang.*) partly separates the sesamoid articular from the cartilage. In the section shown the retro-articular is cut in its membranous part (*m.p.r.A.*), where it is firmly attached to the angular by fibrous tissue (*f.t.*). Other sections show a typical retro-articular process around which the retro-articular is developed as it is in the sardine.

A portion of another section from the same series of T r i g l a is shown at a higher magnification in Text-fig. 16. It includes part of the articular surface of the underlying bone and cartilage. The bone is represented as white, while the strong growth lines and bands are drawn in black. At the articular surface there is a dense layer of fibro-cartilage (*f.c.a.*), and below this is a peculiar tissue which Stephan has shown to be a calcified fibro-cartilage (*c.f.c.*). The cells are rounded and irregularly scattered (*r.c.*),

or elongated and arranged in irregular radiating rows (*e.c.*). These lie in a matrix which stains red with eosin and haematoxylin, not blue as does typical calcified cartilage. At the margin of the formation the matrix passes without interruption
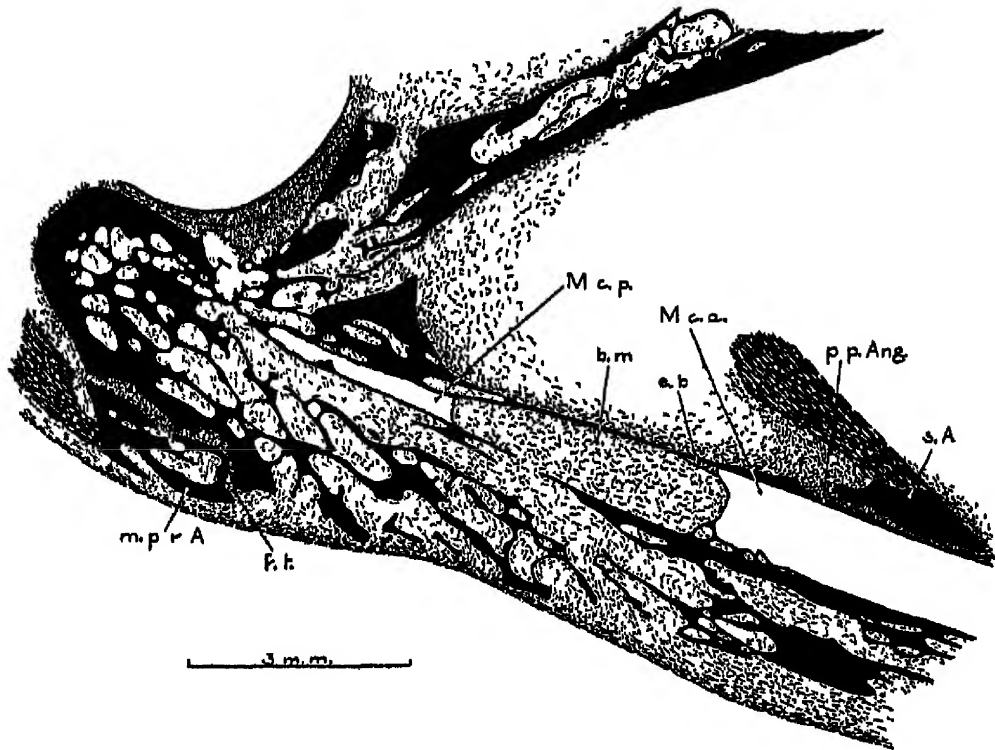
TEXT-FIG. 15.

Longitudinal section of the posterior end of the mandible of Trigla capensis. *b.m.*, bone marrow; *e.b.*, endochondral bone; *f.t.*, fibrous tissue; *M.c.a.*, anterior part of Meckel's cartilage; *M.c.p.*, posterior part of Meckel's cartilage; *m.p.r.A.*, membranous part of retro-articular; *p.p.Ang.*, perichondral part of angular; *s.A.*, sesamoid articular.

into the neighbouring bone. Some of the cells are surrounded by a thin capsule of blue-staining cartilage, others have no such capsule, but all are quite clearly cartilage cells. Thus this tissue consists of cartilage cells embedded in a bony matrix.

As Stephan has pointed out (1900, p. 373), the origin of this tissue can be followed quite clearly at the line of transition from the fibro-cartilage which gives origin to it. The cells of the fibro-cartilage become enlarged and lose their flattened shape, so as to form the rounded or elongated cells of the calcified tissue. The fibres undergo the changes seen in the ossification

Similarly in the Devonian crossopterygian Sauripterus
(Broom, 1913, p. 78) the articular was massive and there was
no retro-articular process. In the Carboniferous amphibian
Orthosaurus (Watson, 1925, 2, p. 214) the articular was
small but very powerful, and there was 'no trace whatsoever
of any post-articular part of the jaw'. In an American Permo-
carboniferous form, however, Trimerorachis (Broom, 1913,
fig. 1), a small retro-articular process projected posteriorly
behind the articular surface, but it is probable that this develop-
ment was quite independent of its development in fishes.

All the forms mentioned above agree in the presence of but
one cartilage bone at the posterior end of the lower jaw, and in
the absence or feeble development of a retro-articular process.
The shape of Meckel's cartilage in these fossil animals is of
course unknown, but it was probably similar to that of Proto-
pterus in having a narrow shaft and an expanded posterior
end. The stimulus which causes bone to form in the peri-
chondrium surrounding a cartilage is unknown, but has probably
something to do with the mass of the cartilage, so that when
a cartilage reaches a certain size critical for the species bone is
laid down around it. In the rod-like cartilages, such as those
forming the branchial apparatus of fishes, bone is first formed
as a girdle round the central part of the rod, leaving the two
ends free of bone so that they can persist as epiphyses. In
Meckel's cartilage the posterior end is much more massive than
the shaft, so that ossification occurs at the end first and the
epiphysial mechanism is not developed. At the anterior end of
the jaw a mento-Meckelian bone may be formed, as in Poly-
pterus (Allis, 1922, p. 247) and Nepatoptychius (Watson,
1928, p. 61).

The detailed structure of the articular in palaeoniscids is still
unknown, but from the shape of the mandible in Elonich-
thys (Watson, 1925, 1, fig. 28) and Nepatoptychius
(Watson, 1928, fig. 11) there can have been little or no retro-
articular development.

In the actinopterygian Polypterus the primitive method
of ossification of the whole of the posterior end of Meckel's
cartilage from a single centre is still found. Here again the bone

is massive and makes firm articulations with the surrounding membrane bones, but it differs from the primitive condition in the development of a long retro-articular process ossified in continuity with the rest of the bone. At the posterior extremity of this process is a cartilage which, though it is not fully differentiated into zones of undifferentiated, flattened, and hypertrophied cells, acts as an epiphysis which directs the growth of the bone. Similar cartilages are found in the same position in modern reptiles. In several lizards (Moodie, 1908) ossified or calcified epiphyses have been described at the posterior end of the jaw, and I have confirmed the presence of cartilaginous epiphyses in this position in the lizard A g a m a a t r a and in the crocodile. Ossification spreading from the main mass of the articular along a narrow retro-articular rod can give rise to the .formation of an epiphysis at the free end of the rod, just as if the retro-articular process were one-half of an ordinary long bone.

A separate centre of ossification for the retro-articular process first appears in the Holostei (Amioidei and Lepidosteoidei), where, in A m i a and L e p i d o s t e u s, a retro-articular bone with membranous, perichondral, and endochondral parts is found (Regan, 1923, 'lower articular', and Mayhew, 1924, 'Angulare'). The membranous part is relatively small, while the endochondral part is large and forms the lower third of the articular surface for the quadrate. In A c e n t r o p h o r u s, the earliest known holostean (Gill, 1923, fig. 5), the bone if present at all had no membranous part and took no part in the formation of the outer surface of the jaw. In E l o p s and some other Clupeiformes (Ridewood, 1904, p. 71) whose retro-articular processes have no special mechanisms of growth, the bone, as in A m i a, helps to form the joint surface, but in most teleosts it takes no part in its formation. An endochondral part may still be developed as in M u g i l, but is usually absent. Thus from its condition in teleosts it would be difficult to say whether the retro-articular was primarily a cartilage or a membrane bone, for both parts may be equally well developed. But as it is traced back to the more primitive forms it becomes clear that it was originally a cartilage bone, and that it has secondarily invaded the membranous parts of the jaw. The stimulus for

the development of a new centre of ossification for the retro-articular process in the Holostei is probably to be found in the development of the process itself. In the young sardine the bone forms a cap in the perichondrium overlying the projecting process, and in Salmo, Amiurus, and Cyclopterus it begins in the same way (Schleip, 1904; Kindred, 1919; Uhlmann, 1921); and there is no reason to expect any difference in the more primitive fishes. Probably in the Holostei the process first reached a sufficient size to induce the perichondrium to lay down bone on its surface, and from the perichondral bone endochondral and membranous extensions have been developed.

In the modern holosteans Amia and Lepidosteus the articular is relatively much smaller than its predecessor in the jaws of Palaeozoic fishes. The last remnant is seen in the Clupeiformes, where in Elops, Megalops, Albula, &c., the articular is still a separate bone (Ridewood, 1904). It has been described here in a young specimen of Elops as a thin plate of bone on the surface of Meckel's cartilage, and later as a larger bone which extends into the cartilage itself. But even in the fully adult fish described by Ridewood the bone is small. In the more typical teleosts the articular has been lost altogether, and the angular has invaded the perichondrium to take its place.

This interpretation of the history of the articular and angular differs materially from that usually advanced. Both the teleostean dentary and the angular are usually spoken of as composite bones (Mischknochen), built up from dermal components, the true dentary and angular, and of ossifications in the perichondrium and cartilage, the mento-Meckelian and articular (van Wijhe, 1882; Schleip, 1903; Kindred, 1915; Goodrich, 1930). Now fusions between the articular and neighbouring bones do actually occur in the development of several amphibians and reptiles (Gaupp, 1911, 'Gonio-articulare', and Eifertinger, 1933, 'Pseudangulare'). But at no time in the actual development of any teleost have two separate ossifications been seen. Schleip in Salmo has shown that the dentary begins as a single continuous plate of bone, which at the extreme anterior end of Meckel's cartilage lies in the perichondrium itself, and more posteriorly is separated by a layer of connective

tissue from the cartilage, while the angular begins as a similar plate which lies entirely outside the perichondrium. In both bones extensions grow from the original plate into the perichondrium and into the connective tissues. In Amiurus (Kindred, 1915) the dentary at first lies entirely outside the perichondrium, and only later invades it, while the angular begins in the perichondrium and later extends into the connective tissues. In Cyclopterus (Uhlmann, 1921) both the dentary and the angular are formed as is the dentary of Salmo, partly in and partly outside the perichondrium. Thus in these three fishes the dentary may be laid down outside the perichondrium or partly within it, the angular outside, partly within, or entirely within the perichondrium. Yet there can be no doubt that the dentary and angular are homologous in all three, so that little importance can be attached to the developmental origin of a teleostean bone, a point emphasized by Schleip in his consideration of the vomer, an undoubted membrane bone which in Salmo arises entirely within the perichondrium.

Further, the process of invasion of the perichondrium by a membrane bone can be followed in the sesamoid articular of various teleosts. It may retain its more primitive position in the connective tissues (Mugil, Trigla), or invade the perichondrium (Elops), or may even present the appearance of a typical cartilage bone, completely surrounding Meckel's cartilage, constricting it and including a localized calcification (Sardina). I have never observed endochondral bone in association with the sesamoid articular, but there is no theoretical reason why the calcified cartilage should not be replaced by bone and marrow. Though it may still be possible to say that cartilage bones are always related to cartilage in development, or sometimes to unchondrified parts of the primordial skeleton, no such generalization can be made about membrane bones in teleosts.

Now the mento-Meckelian ossification is, when present, always a small bone, while the articular, though an important bone in primitive fishes, has already become small and has lost its firm articulations with neighbouring bones in Holosteans.

It seems, however, more likely that these bones have disappeared in teleosts than that they have become expanded to form the extensive perichondral ossifications found in association with the dentary and angular. Further, Uhlmann (1931) has shown the difficulty of adopting the 'Mischknochentheorie' to account for the membranous and perichondral parts of the dentary and angular on embryological grounds. The theory that the dentary and angular are simple bones of membranous origin, that have in teleosts invaded the periosteum and cartilage, seems best to fit the known facts of structure and development.

The medio-Meckelian ossification, discovered by Schmäh (1934) in Polypterus, is as far as is known peculiar to that fish, though an extension from a similar bone may have been responsible for the complete ossification of Meckel's cartilage found in Sauricthys by Stensio (quoted by Goodrich, 1930, p. 403).

In the typical teleosts, after the membranous angular has invaded the perichondrium, the cartilage is ossified from this new perichondral bone just as it was from the old. But the cartilage, having lost its importance as a matrix for a large articular bone, remains small compared to the size of the mandible, the only expanded parts being the articular cartilage and the retro-articular process which are retained, the one as a bearing surface of the joint, and the other as a basis for the angular. The individual specializations have been described, and need not be further discussed here.

The history of the sesamoid articular has been disputed. The relation which it always bears to the tendon of the adductor mandibulae gave rise to the suggestion that it is a sesamoid structure (Ridewood, 1904; Starks, 1916), but sesamoid bones are not usually developed in fishes. Regan (1913) suggested that it was a reduced pre-articular, but in Amia there is a sesamoid articular quite separate from the pre-articular, represented by the so-called splenial. Schleip (1904) from its development in Salmo described it as a cartilage bone, but it is clear from its condition in such fish as Mugil and Trigla that it has no necessary connexion with the perichondrium. The discovery of a specially ossified part of the angular in Poly-

pterus in a position exactly comparable to that of the sesa-
moid articular in Holostei and Teleostei makes it probable that
these two structures are homologous. The invasion of the
perichondrium sometimes seen in Teleostei, as in Elops and
Sardina, is clearly secondary, and can be compared to in-
vasion by the derm-articular.

SUMMARY.

1. In modern Dipnoi (Protopterus) the membrane bones
are separated by connective tissue from Meckel's cartilage, and
there is no endochondral or perichondral bone. The cartilage
grows evenly over its whole extent.

2. In Polypterus a large articular ossifies the posterior
end of the cartilage, including the retro-articular process, and
spreads into the neighbouring connective tissues.

3. In Elops the joint surface is carried partly by the arti-
cular, and partly by the retro-articular, a special ossification of
the retro-articular process.

4. In most teleosts (Mugil, Sardina, Trigla) the arti-
cular is absent and the angular invades the perichondrium and
cartilage to form the joint surface. Special growth zones of
flattened cells are formed in the cartilage which by their growth
carry the retro-articular, angular, and dentary away from one
another, stability of the jaw being maintained by new growth
of the membranous parts of the bones.

5. Endochondral bone is reduced or absent in some specialized
fishes (Tetrodon, Notopogon).

6. The sesamoid articular of teleosts is a separated part of the
angular which gives insertion to the adductor mandibulae
muscle.

7. An attempt is made to follow the evolution of Meckel's
cartilage and the related ossifications by a comparison of the
early Dipnoi, Crossopterygii, and Amphibia described in the
literature with modern forms.

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The Musculature of the Mouth-parts of Insect Larvae.

By

G. M. Das, M.Sc., D.I.C.

(Department of Entomology, Imperial College of Science and Technology, London.)

With Plates 1–12.

Contents.

1. Introduction.

Various papers have been published on the morphology of the mouth-parts of adult and larval insects, but there still exist great discrepancies in the interpretation of the sclerites and lobes of the mouth-parts, especially of the labium.

Not only are the sclerites of the labium misinterpreted by many entomologists, but the real homology of the sclerites

when compared with those of the maxilla has not been properly understood.

Comparative study of the skeletal structure alone fails in certain cases to give a correct interpretation of the sclerites, lobes, &c., of the mouth-parts, but the musculature seems to be a much safer guide. Snodgrass (1935) has tried to clear up certain difficulties on the basis of musculature, but left many of the controversial points still unsettled.

In view of this, and also to find out the real homologies of the sclerites, I have studied the musculature of the mouth-parts of larval insects prior to the study of adults.

The conclusions put forward here are based on the study of about thirty species of larval insects.

2. MATERIAL AND TECHNIQUE.

The material was fixed in 90 per cent. alcohol for three to ten days, according to the size of the larva and the nature of its food. The fixed material could be kept for a long time without distortion of the muscles, if an incision was made in the thorax or if the head with a part of the thorax was severed from the body.

The dissections were made in 70 per cent. alcohol and the dissected mouth-parts with their muscles were stained overnight in light borax carmine. The stain was removed from the sclerotized parts in 70 per cent. alcohol. Permanent preparations were made of the mouth-parts with their muscles intact.

To study the details of the mouth-parts, the specimens were boiled in weak caustic potash solution for a short time. In order to get rid of muscles easily, they were treated with boiling water for some time, prior to the boiling in caustic potash.

In a few cases to verify certain controversial points, microtome sections were cut and the parts reconstructed.

The following larval insects were studied:

Coleoptera.

1. Tenebrio molitor, L. . . . } Tenebrionidae
2. Tenebrio obscurus, F. . . }

 3. Ptinus tectus, Boield . . . Ptinidae
 4. Dermestes vulpinus, F. . . Dermestidae
 5. Tenebroides mauritanicus, L.. Trogositidae
 6. Thanatophilus sp. . . . Silphidae
 7. Agriotes sp.. . . . Elateridae
 8. Rhagium sp.. . . . Cerambycidae
 9. Hydrophilus piceus, L. . . Hydrophilidae
10. Sinodendron cylindricum, L. .} Lucanidae
11. Lucanus cervus, L. . . .}
12. Calandra granaria, L. .
13. Caulophilus latinasus, Say .} Curculionidae
14. Ceuthorrhynchus pleuro-
      stigma, Marsh. . . .
15. Carabus sp. . . . . Carabidae

Neuroptera–Megaloptera.

16. Sialis lutaria, L.
17. Raphidia sp.

Hymenoptera.

18. Pteronidea ribesii, Scop. . . Tenthredinidae

Mecoptera.

19. Panorpa communis, L.
20. Panorpa germanica, L.

Lepidoptera.

21. Clisiocampa neustria, L. . . Lasiocampidae
22. Galleria mellonella, L. . Galleridae
23. Agrotis sp. . . . . Noctuidae

Trichoptera.

24. Anabolia nervosa, Leach . . Limnophilidae
25. Hydropsyche sp.. . . . Hydropsychidae
26. Hydroptila sp. . . . . Hydroptilidae
27. Philopotamus sp. . . . Philopotamidae

Diptera.

28. Tipula flavolineata, Mg. . . Tipulidae
29. Bibio sp. . . . . . Bibionidae

In addition some other larvae were also dissected for certain special points. The following adult types were also examined.

1. Periplaneta americana, L.
2. Tenebrio molitor, L.
3. Chrysopa sp.
4. Panorpa communis, L.
5. Boreus hiemalis, L.
6. Termopsis sp.

The work was carried out in the Imperial College of Science and Technology under the direction of Prof. J. W. Munro. I am very much indebted to Dr. O. W. Richards for valuable suggestions and kind criticism throughout the investigation. My thanks are also due to other members of the staff and to Messrs. N. Ahmad and J. A. Downes for friendly assistance.

3. THE LABRUM.

The labrum or upper lip (figs. 1–8, *lm.*, Pl. 1; figs. 9–11, *lm.*, Pl. 2) of insects is typically a broad plate at the ventral margin of the cranium. It commonly overlies the mandibles and is suspended from the clypeus. Its posterior (inferior) wall, which is called the epipharynx (fig. 10, *ep.*, Pl. 2), is provided with gustatory papillae.

It varies much in shape and size in adult insects, but in the larval insects it is usually a broad flap which is roughly elliptical or quadrate with or without a notch at its anterior margin.

In larval Carabidae and Elateridae it seems superficially to bo absent, but a careful examination shows that it is drawn towards the epipharyngeal side (fig. 4, *lm.*, Pl. 1). The musculature, however, reveals its true identity. In larval Hydrophilus it is absent, unless fused with the hard sclerite at the proximal margin of the head, which is most probably the clypeus.

On the lateral angles of the labrum there is a pair of darkly pigmented sclerites, called the tormae (figs. 1, 2, 3, 8, *t.*, Pl. 1; fig. 9, *t.*, Pl. 2). They extend a considerable distance on the epipharyngeal side of the clypeus and may run even to the frontal region (fig. 5, Pl. 1). Snodgrass (1931) regards these processes as the landmark of the proximal limit of the labrum,

but in the larvae of Curculionidae they are situated entirely on the epipharyngeal surface, their distal end being near the anterior margin of the labrum (fig. 2, *t.*, Pl. 1). In certain larval insects such as Panorpa and Thanatophilus they are totally absent (fig. 7, Pl. 1 ; fig. 10, Pl. 2).

The tormae are sclerotized rods on which the labral muscles (*3*) from the frons (*f.*) are inserted.

The musculature of the larval labrum comprises the following muscles (figs. 1–8, Pl. 1 ; figs. 9–11, Pl. 2): ·

(1) A group of small fibres joining the anterior and posterior walls of the labrum.

(2) Anterior labral muscles—arising on the frons, inserted on the anterior walls of the labrum.

(3) Lateral labral muscles—arising on the frons, inserted on the tormae.

(4) Posterior labral muscles—arising on the frons, inserted on the posterior wall of the labrum, i.e. on the middle of the epipharyngeal side of the labrum.

The muscles joining the anterior and posterior walls of the labrum are present in many larvae, but it is very difficult to detect them owing to their small size. All of the muscles that arise on the frons are not present in the same larva, either one or two sets may be absent. In larval Neuroptera, Trichoptera, and Hymenoptera (figs. 1, 3, Pl. 1 ; figs. 9, 11, Pl. 2) both anterior and lateral muscles (*2, 3*) are present, while in larval Thanatophilus (fig. 10, Pl. 2) and Lucanidae only the posterior muscles (*4*) are retained. In all larval Lepidoptera (fig. 8, Pl. 1) and most Coleoptera (figs. 2, 5, 6, Pl. 1), only the lateral muscles (*3*) are present.

In larval Carabidae and Elateridae the labrum has been drawn towards the epipharyngeal side. In the former (fig. 4, Pl. 1) both anterior and posterior walls are represented by two sclerites on the same side, the distal sclerite (*lm.*) representing the anterior wall, and the proximal sclerite (*eph.*), to which the posterior muscles (*4*) are attached, the posterior (epipharyngeal) wall of the labrum. In larval Elateridae the labrum is likewise situated on the epipharyngeal side, closely apposed to the

clypeus, but here it is a semilunar bar fringed with hairs and provided with tormae to which the lateral muscles from the frons are inserted. The origin of these muscles upon the frons, and their insertion upon the tormae, suggest the identity of the labrum.

All muscles (2, 3, 4) of the labrum except those joining the anterior and posterior walls have their origin on the frons (figs. 1–8, Pl. 1; figs. 9–11, Pl. 2). In certain cases (figs. 3, 5, 6, Pl. 1) when only the lateral muscles (3) are retained they arise on an internal ridge of the frons.

The origin of the labral muscles, according to Snodgrass (1928, 1931, 1935), identifies the frontal sclerite in all insects with the exception of the adult Diptera in which they have their origin upon the clypeus. In larval Lepidoptera (fig. 8, Pl. 1) the labral muscles (3) arise on a median ridge (fr.) behind the triangular plate (cl.). This ridge, according to Snodgrass, is the inflected part of the frons and the so-called frons in lepidopterous larvae is really the clypeus (cl.).

4. The Hypopharynx.

The hypopharynx (figs. 50, 55, hph., Pl. 10; fig. 57, hph., Pl. 11) is a tongue-like median projection arising on the floor of the mouth between the bases of the mouth-appendages. According to embryologists it is formed from the sterna of the mandibular and first maxillary segments, but some authors suppose that a part of the sternal element of the second maxillary segment has been incorporated.

In the larval insects the hypopharynx becomes fused with the dorsal surface of the labium and in certain larvae (e.g. in Trichoptera, Lepidoptera, and Hymenoptera) it may become fused with the ligula so as to form a composite structure (figs. 50, 52, sp., Pl. 10) bearing the orifice of the labial glands. In many larvae it lies proximally to the dorsal surface of the prementum just behind the insertion of the dorsal muscles of the prementum.

On the sides of the hypopharynx are a few strongly sclerotized plates, called the suspensoria (figs. 57, 58, cl., Pl. 11), which support the hypopharynx and on which the muscles from the frons

are inserted. According to Snodgrass (1935) the suspensorial area should not be regarded as a part of the hypopharynx, and details of this structure and of its muscles are not dealt with here. A pair of muscles (5) arising on the tentorial bridge (figs. 41, *tb.*, Pl. 8; 52, 55, *tb.*, Pl. 10) are sometimes inserted on the hypopharynx.

The insertion of the dorsal premental muscles is very helpful in determining the anterior limit of the hypopharynx. In the larvae of Thanatophilus (fig. 24, Pl. 4) the dorsal surface of the prementum (*prm.*) simulates the hypopharynx, but the insertion of the dorsal muscles (21) at its posterior region shows its true nature.

In certain insects (e.g. larval Lucanidae) the hypopharyngeal region has become highly sclerotized, forming a structure like the molar area of the mandible. In the larvae of Tenebrio only the posterior region of the hypopharynx forms such a structure, but it is supported on the hypopharyngeal bracon—a rod attached to the hypostomal lobes of the head capsule near the mandibular articulations. The hypopharyngeal bracon is developed in many cases, even when the hypopharynx remains membranous. In the hypopharynx of larval Dascillus, Carpenter and MacDowell (1912) describe a similar structure which works against teeth on the epipharyngeal surface of the labrum. The present author could not find such teeth on the epipharynx of the larvae of Lucanidae, but there are two strongly sclerotized plates to which the posterior muscles of the labrum are attached.

The superlinguae are a pair of lateral lobes attached to the hypopharynx. Following the interpretation of Hansen (1893) the superlinguae have been homologized with the maxillulae of Crustacea by many authors, e.g. Carpenter (1903), Evans (1921), and Henriksen (1928), but Crampton (1921) contradicts this view, believing that they correspond to the paragnaths of Crustacea. No muscles, however, could be found at the bases of the superlinguae so that the question must be decided on other evidence.

The superlinguae in larval Tenebrio are very well developed, with fine hairs at their distal borders.

5. The Mandibles.

The mandibles (figs. 12–19, Pl. 2) are a pair of strongly sclerotized jaws, situated on each side of the mouth immediately behind the labrum. They represent the basal segment or coxopodite of the typical Arthropod limb (Crampton, 1921). According to the nature of the food and the mode of feeding, they are highly modified into biting, chewing, sucking, and piercing organs. They may be functionless or even completely absent.

In larval insects the mandibles usually preserve the generalized biting and chewing type of structure (fig. 13, Pl. 2). Each mandible has a broad triangular base, a mesal surface produced into incisor lobes (in.), and a molar or crushing surface (mo.) near the base. In many cases the right and left mandible differ in the number of incisor points.

The mandibles are attached to pleurostomal margin of the cranium by the outer edge of the triangular base and have an articulation with the head at each end of the hinge line. Anteriorly they are articulated by means of a ginglymus or groove (figs. 14, 15, g., Pl. 2) which fits into a convex process of the head, and posteriorly by means of a condyle (c.) which fits into a socket at the lower margin of the gena or post-gena.

In the predacious larval forms, however, the mandible loses its crushing area, while its incisor surface becomes very sharp and may be toothed. In the larvae of Sialidae, Raphidiidae, Carabidae, &c., the mandibles are elongated with sharp points but without any molar area (figs. 15, 19, Pl. 2). In the larvae which feed on the juices of their prey the mandibles exhibit a special modification for sucking. In the predacious larvae of Chrysopidae each mandible (fig. 16, Pl. 2) is fang-shaped and along the entire length of the ventral surface is a groove (gr.) which fits against the maxilla, thus forming a channel through which the juices of the prey can be sucked. In the larvae of Dytiscus (Rungius, 1911; Korschelt, 1924) the mandibles are also fang-like, each being traversed by a canal through which the poison from the stomach is injected into the body of the prey, dissolving the soft body-tissue. The liquefied emulsion thus formed is sucked in through the mandibles by a special mechanism involving the other mouth-parts (Blunck, 1916).

In many insects there is a brush-like process fixed to the inner side or base of the mandible. This process is called the penicillus (fig. 17, Pl. 2). In a few cases a movable plate fringed with hairs is articulated at the inner base of the mandible. This is called the prostheca or lacinia mobilis (fig. 18, *pr.*, Pl. 2). The prostheca is incorrectly homologized with the lacinia. The projecting terminal lobe of the mandible, according to Snodgrass (1934), is an endite of the basis; he states, 'in Diplopoda this lobe is freely movable and in both diplopods and chilopods it is provided with muscles corresponding to the muscles of the lacinia of a generalized insect maxilla. In other groups the terminal lobe loses its mobility and becomes solidly fused with the basis, in consequence of which its muscles have disappeared.' The lacinia mobilis is present in a well-developed condition in the larva of Diptera (Tipulidae and Bibionidae), where it is attached to a flexible area at the inner base of the mandible. There is no muscle inserted upon it.

Each mandible in larval insects is moved by means of powerful abductor and adductor muscles (figs. 12, 17, Pl. 2).

(6) Abductor muscle.—A small muscle arising on the lateral wall of the cranium and inserted on a small apodeme, attached to the outer margin of the mandibular base.

(7) Adductor muscle.—This is a very large muscle arising on the dorsal and posterior wall of the cranium and inserted on a large apodeme at the inner angle of the mandibular base.

These two muscles (abductor and adductor) represent the dorsal muscles of the generalized insect limb, but the ventral muscles of such a limb are absent in the mandible of most larval insects. They are, however, stated to be present in well-developed condition in the early stages of Ephemeroptera and Odonata, having their origin on the tentorium. With the increased size of the dorsal muscles, the ventral muscles become of secondary importance and are usually reduced or absent (Snodgrass, 1935).

6. The Maxillae.

The maxillae (fig. 62, Pl. 12) of an insect are the appendages of the fifth cranial segment, each having the typical structure of

a limb with a basal shaft, two lobes, and a telopodite. The basis is composed of a proximal sclerite, the cardo (*cd.*), and a distal sclerite, the stipes (*st.*). The cardo is attached to the head by the pleural membrane and articulated to the hypostomal margin of the cranium by a small condyle (*c.*) The inner side of the cardo near the condyle is inflected so as to form a process (*p.*) to which the promotor muscle (*8*) of the cardo is attached. Crampton (1925) calls this the cardo-process. Running from the cardo-process in many insects there is an internal ridge which subdivides the cardo into a proxicardo and a disticardo. The stipes (*st.*) at the distal end bears two lobes. The inner lobe is the lacinia (*lc.*) and the outer is the galea (*ga.*). Lateral of the galea is the maxillary palpus or telopodite (*mp.*). The lateral area of the stipes bearing the palpus is sometimes differentiated as a small sclerite called the palpifer. The number of segments in the palpus varies.

The maxilla is attached at its base to the labium by an articulating membrane, the basi-maxillary membrane (fig. 24, *bm.*, Pl. 4). Occasionally the articulating area is strongly sclerotized, forming a definite sclerite or sclerites, called the basimaxillary sclerites (fig. 29, *msc.*, Pl. 5).

The maxilla in most larval insects has the typical structure mentioned above, but in a few cases it suffers a great reduction and loss of component parts. The suppression of one or both lobes is very common.

In larval insects the cardo (fig. 23, *cd.*, Pl. 3; fig. 24, *cd.*, Pl. 4; fig. 34, *cd.*, Pl. 6; figs. 41, 44, *cd.*, Pl. 8) is generally triangular in shape, articulated to the head by the condyle. In larval Rhagium (fig. 23, Pl. 3), however, it is devoid of a condyle, but attached to the head by the pleural membrane only. In larval Diptera and in certain Trichoptera it is rather rod-shaped (fig. 45, Pl. 9; figs. 56, 59, 60, Pl. 11).

The cardo in many larval insects is subdivided by an internal ridge which strengthens the sclerite but rarely gives insertion to the tentorial adductors of the cardo. With the absence of promotor muscle the cardo loses its cardo-process. The latter is absent in larval Diptera, Trichoptera, Lepidoptera, Mecoptera, and also in certain Coleoptera such as larval Elateridae and

Trogositidae (figs. 28, 29, Pl. 5; fig. 40, Pl. 7; fig. 46, Pl. 9; figs. 54, 55, Pl. 10; figs. 56, 60, Pl. 11).

The two cardines in larval Elateridae have become mesally approximated; consequently, with the displacement of the post-mentum (fig. 31, *pmt.*, Pl. 5) forward there is left only a suture between the two cardines.

The direction of the cardo varies with the prognathous and hypognathous conditions of the head. In typical prognathous larval insects (figs. 28, 31, Pl. 5; fig. 37, Pl. 7; fig. 41, Pl. 8) it runs straight forward, while in the typical hypognathous type (fig. 30, Pl. 5; fig. 34, Pl. 6) it is directed outwards and forms an angle with the stipes. With the reduction in size of the labium in larval Mecoptera, the distal end of the cardo is turned inwards (fig. 40, Pl. 7).

The stipes is suspended from the cardo in the hypognathous type. In the prognathous type it runs straight forward in the same line as the cardo.

Yuasa (1920) states that the stipes has two points of articulation with the cardo in Orthopterous insects. The same condition exists in most larval insects, but in larval Diptera the cardo (figs. 56, 59, 60, *cd.*, Pl. 11) is attached to the stipes by a single articulation.

The distal area of the stipes bearing the palpus is sometimes differentiated into a distinct sclerite, the palpifer (fig. 37, *pf.*, Pl. 7), but in many cases the area remains membranous (fig. 45, Pl. 9). The basal segment of the palpus in many insects is misinterpreted as the palpifer.

The lacinia in most larval insects is spined or toothed[1] on its inner border. The galea is sometimes two-segmented as in larval Carabidae and Elateridae (figs. 29, 31, *ga.*, Pl. 5). In certain cases the galea is partially fused with the basal segment of the palpus. When this happens the outer part of the compound structure is sometimes mistaken for the palpifer. An examination of the palpal muscles would reveal its true identity.

In the larvae of S i a l i s the lobe (fig. 41, *ga.*, Pl. 8), apparently borne by the basal segment of the palpus, is undoubtedly a part of the galea. The unusually dilated basal segment (*b.*)

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[1] Teeth not shown in many figures.

of the palpus tends to show that the galea has most probably two segments, the proximal segment being fused with the basal segment of the palpus, while the distal one remains free. The musculature also supports this view.

In the larvae of Raphidia, Panorpa, Thanato-philus, and Ptinus the lacinia and galea are fused to form a single structure, but its distal end remains notched, thus revealing the presence of both lobes (figs. 24, 25, *lc.*, *ga.*, Pl. 4; fig. 34, *lc.*, *ga.*, Pl. 6; figs. 37, 39, 40, *lc.*, *ga.*, Pl. 7). In larval Raphidia (fig. 40, Pl. 7) the presence of stipital flexors (*13, 14*) of the lacinia and galea further supports this view.

In many larvae either one or both lobes may be suppressed. In the larvae of Trichoptera, Diptera, Curculionidae, Tenebrio, and Tenebroides, only the lacinia (figs. 20, 21, *lc.*, Pl. 3; fig. 28, *lc.*, Pl. 5; fig. 32, *lc.*, Pl. 6; figs. 45, 46, *lc.*, Pl. 9; figs. 56, 59, 60, *lc.*, Pl. 11) is present, while in the larvae of Cerambycidae and Carabidae[1] only the galea (fig. 23, *ga.*, Pl. 3; fig. 29, *ga.*, Pl. 5) is retained. Both lobes are totally absent in larval Hydrophilus (fig. 27, Pl. 4), and the lobe (*el.*) borne by the basal segment of the palpus is the endite of that segment.

In Lepidopterous larvae (figs. 50, 51, 54, 55, Pl. 10) both lacinia and galea are absent. The lobe (*l.*) borne by the second segment of the palpus can in no way be regarded as either lacinia or galea. It is most probably a secondary outgrowth of the palpal segment.

The palpus is variously segmented. In larval Diptera it is composed of a single segment (figs. 56, 59, 60, *mp.*, Pl. 11).

The detailed identities of the sclerites and lobes will be dealt with in relation to musculature.

7. The Musculature of the Larval Maxillae.

The musculature of the typical larval maxillae comprises the following muscles (figs. 20, 21, 23, Pl. 3; figs. 24, 25, 27, Pl. 4; figs. 28, 29, 30, 31, Pl. 5; fig. 34, Pl. 6; figs. 39, 40, Pl. 7; figs. 41, 44, Pl. 8; fig. 46, Pl. 9; figs. 54, 55, Pl. 10; figs. 56, 60, Pl. 11; fig. 62, Pl. 12).

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[1] The spine-like process (*lc.* ?) on the mesal side of the stipes may represent the remnant of the lacinia.

(8) Promotor of the cardo.—A group of fibres, generally arranged in a fan-shaped manner; origin on the postgena, insertion on the cardo-process.

(9) Adductors of the cardo.—Generally two muscles; origin on the tentorium, insertion of one on the lateral side, of the other on the mesal side of the cardo. When the cardo is subdivided, one is inserted on the proxicardo and the other on the disticardo.

(10) Adductor of the stipes.—Origin on the tentorium, insertion either in one or in two groups on the stipes.

(11) Retractor of the stipes.—Origin on the basal part of the tentorium, insertion on the stipital ridge.

(12) Cranial flexor of the lacinia.—Origin just dorsal to the origin of the promotor of the cardo, insertion on the inner base of the lacinia.

(13) Stipital flexor of the lacinia.—Origin on the outer angle of the stipital base, insertion on the inner base of the lacinia near the insertion of the cranial flexor. In many cases the stipital flexor and cranial flexor of the lacinia have a common insertion through a tendinous cord.

(14) Cranial flexor of the galea.—Origin on the mesal side of the stipes, insertion on the base of the galea.

(15) Levator of the maxillary palpus.—Origin on the median basal part of the stipes, insertion on the dorsal margin of the basal segment of the palpus.

(16) Depressor of the maxillary palpus.—Origin near the origin of the levator, insertion on the ventral margin of the basal segment of the palpus.

The additional muscles of the larval maxilla (fig. 40, Pl. 7; fig. 46, Pl. 9; fig. 54, Pl. 10; figs. 56, 60, Pl. 11):

(17) Cranial flexor of the maxillary palpus.—Origin on the hypostoma in larval Lepidoptera or dorsal region of the postgena in larval Trichoptera; insertion near the levator of the maxillary palpus.

(18) Cranial flexor of the stipes.—Origin on the gena, insertion on the anterior (dorsal) side of the stipes; present in larval Panorpa and Bibio.

8. The Musculature in Relation to the Sclerites, Lobes, etc., of the Larval Maxilla.

The cardo can be distinguished by the insertion of the pro-motor (*9*) and adductor muscles (*10*) upon it. The adductors of the cardo are generally two groups of fibres arising on the tentorium. One group is inserted on the outer side and the other on the mesal side of the cardo (fig. 23, Pl. 3), or when the cardo is subdivided by the internal ridge each subdivision has the insertion of one group (fig. 20, Pl. 3). Rarely, the internal ridge itself has the insertion of a few fibres. In larval Elateridae, Crampton (1928) calls the disticardo (fig. 31, *dc.*, Pl. 5) the basimaxillary sclerite. The insertion of the tentorial adductors of the cardo on both sclerites, labelled *pc.* and *dc.*, shows that they are subdivisions of the cardo, and the sclerite, *dc.*, cannot be a basimaxillary sclerite which always lacks muscles.

In larval Lepidoptera the sclerite labelled *msc.* in fig. 50, Pl. 10, lying on the mesal side of the cardo is called the sub-mental sclerite by Crampton (1921, 1928), but Snodgrass (1928) labels it as the accessory plate of the cardo. The sclerite is separated by suture from the postmentum and has no insertion of any muscles upon it. The above condition suggests that it is neither a part of the postmentum nor of the cardo, but from its position I am inclined to regard it as the basimaxillary sclerite of the same nature as that of larval Carabidae (fig. 29, *msc.*, Pl. 5) and Tenebrionidae.

In many larval insects the cardo is devoid of its promotor muscle and in larval Mecoptera and Diptera it entirely lacks muscles (fig. 40, *cd.*, Pl. 7; figs. 56, 60, *cd.*, Pl. 11).

The stipes (fig. 63, *st.*, Pl. 12) can be distinguished by the origin of the muscles of the palpus (*15, 16*) and of the lobes (*13, 14*). The insertion of the adductor (*10*) and retractor (*11*) of the stipes is very helpful in finding the mesal limit of the stipes (fig. 20, Pl. 3; fig. 25, Pl. 4; fig. 34, Pl. 6). The dorsal cranial muscle (*18*) of the stipes, in the absence of other muscles, is the last resource for the interpretation of the sclerite (fig. 40, *st.* Pl. 7; fig. 60, *st.*, Pl. 11) from the point of view of musculature. This muscle is most useful in interpreting the stipes in larval Bibionidae (fig. 60, *st.*, Pl. 11).

In many insects the stipes has on the mesal side a small sclerite, apparently demarcated from the main one. This is mainly due to the insertion on that area of the tentorial adductor and the retractor of the stipes.

The lacinia (fig. 30, *lc.*, Pl. 5; fig. 39, *lc.*, Pl. 7; fig. 62, *lc.*, Pl. 12) has a cranial flexor (*12*) and a stipital flexor (*13*), and the galea has only the stipital flexor (*14*). In adult Orthoptera (fig. 62, Pl. 12) the stipital flexor of the lacinia (*13*) lies dorsal (anterior) to the palpal muscles (*15, 16*), and the flexor of the galea (*14*) runs ventral (posterior) to the palpal muscles. The same condition exists in many adult insects and most larval stages, but in the latter the flexor of the galea arises near the origin of the palpal muscles or rather on their mesal side (fig. 23, Pl. 3; fig. 30, Pl. 5; fig. 40, Pl. 7, fig. 41, Pl. 8). These muscles are very important in distinguishing the lobes. The flexor of the galea is not present in many larval stages, but when the lacinia is present, its muscles, or at least the cranial flexor, are always retained. The cranial flexor of the lacinia undoubtedly plays an important part in interpretation of the lobes or of the lobe when one of them is absent; even in the absence of the lacinia as in larval H y d r o p h i l u s (fig. 27, Pl. 4) the cranial flexor of the lacinia (*12*) is retained, but its point of insertion is changed, being removed to the dorsal middle of the stipital base.

Otanes (1922) describes the two lobes in adult Mecoptera as subdivisions of the galea, the lacinia being completely absent. In view of this, and to study the modification, I have also examined adult P a n o r p a and B o r e u s and found that the lobes were definitely the lacinia and galea, since the typical muscles of the lacinia are retained in the former lobe.

The palpus has a levator (*15*) and a depressor muscle (*16*) arising upon the stipes (fig. 25, Pl. 4; figs. 28, 31, Pl. 5; fig. 34, Pl. 6; fig. 54, Pl. 10; fig. 62, Pl. 12). An accessory muscle (*17*) of the palpus arising on the cranium is found in larval Trichoptera and Lepidoptera (fig. 46, Pl. 9; fig. 54, Pl. 10). This muscle is not found in any other group. In larval Diptera the palpal muscles are absent (figs. 56, 60, Pl. 11).

The maxilla of larval Diptera suffers a great loss in its mus-

culature, but it retains most of its component parts in reduced condition. The only muscle present in larval Tipulidae (fig. 56, Pl. 11) is the cranial flexor of the lacinia (*12*) which controls the whole maxilla; in larval Bibio (fig. 58, Pl. 11) there is an additional muscle, the dorsal muscle of the stipes (*18*).

The maxilla in larval Diptera (figs. 56, 59, 60, Pl. 11) has a rod-shaped cardo (*cd.*) articulating with the hypostoma, a stipes (*st.*), a lacinia (*lc.*), and a single segmented palpus (*mp.*).

It is quite improbable that the palpifer represents a segment of the mouth-part limb with the lacinia as its endite, as is claimed by Hansen (1893, 1930) and Crampton (1925). The musculature does not provide any evidence for this view. Borner (1921) and Snodgrass (1928) regard the palpifer as a secondarily demarcated portion of the stipes. The latter author states, 'that the palpifer is not a segment of the appendage is shown by the fact that muscles neither arise within it nor are inserted upon it'. The origin of the muscles of the palpus and of the flexor of the galea upon the stipes adds much weight to the view of Borner and Snodgrass. On the other hand, the origin of the muscle of the galea upon the stipes shows that the galea cannot be an endite of the so-called palpiferal segment, but it belongs to the stipes.

In support of his view, Crampton (1925) cites the larva of Sialis and through it he tries to bridge the gulf between Crustacea and Insecta and to show the modification of the 'palpiferal segment'. He calls the segment *b*, in Sialis larva (fig. 41, Pl. 9), the palpifer, representing the ischiopodite of the Crustacean appendage, and its endite, the galea. An examination of the musculature, however, shows that the typical palpal muscles (*15, 16*) are inserted at the base of this segment (*b.*), which is undoubtedly the basal segment of the palpus. Snodgrass has also interpreted the segment on the basis of musculature. The inner lobe (*ga.*), apparently borne by the basal segment of the palpus, is called the galea by Crampton, on the assumption that the segment labelled *b.* is the palpifer, but according to Snodgrass it is an endite lobe of the first segment of the palpus. The latter author criticizes Crampton, stating that 'it cannot be galea since it lacks muscles'. On this

point I do not agree with Snodgrass's interpretation, for the galea in many larval insects has been found to be without muscles. Besides, in larval S i a l i s the lobe undoubtedly has a muscle (*14*) inserted at its apparent base (this has been confirmed by cutting sections and reconstructing). This muscle is homologous with the flexor of the galea (*14*) of other larval insects (fig. 23, Pl. 3; fig. 30, Pl. 5; fig. 39, Pl. 7) and has a similar origin; but in S i a l i s larva it passes through the basal segment of the palpus to be inserted on the apparent base of the lobe (fig. 41, *ga.*, Pl. 8). The unusually dilated basal segment of the palpus, and the insertion of the typical muscles of the galea on the apparent base of the lobe, tend to prove that the galea in S i a l i s larva like that of the Carabidae and Elateridae (figs. 29, 31, *ga.*, Pl. 5) is two-segmented, but the proximal segment has become fused with the basal segment of the palpus, while the distal segment remains free.

In larval Trichoptera (figs. 45, 46, Pl. 9) the lobe (*lc.*) is the lacinia. The cranial flexor of the lacinia (*12*) is inserted on its inner base, but the stipital flexor of the lacinia is absent. Crampton (1928) calls the lobe (*lc.*) the galea, while Belton (1934) suggests it is the fused lacinia and galea. The lobe does not show any sign of external demarcation as is found in the fused lacinia and galea of larval T h a n a t o p h i l u s, P a n o r p a, and R a p h i d i a and is, therefore, probably the lacinia only.

In Lepidopterous larvae (figs. 50, 51, 54, 55, Pl. 10), Snodgrass (1928) suggests that the entire structure (*mp.*) distal to the stipes (*st.*) is the lacinia. The insertion of the palpal muscles (*15, 16*) at the base of the structure shows that it is the maxillary palpus, and the lobe (*l.*) borne by the second segment of the palpus cannot be the galea as it was supposed to be by Crampton (1921). For the galea could not be borne by a distal segment of the palpus, unless it is proximally fused with the two basal segments of the palpus, which is improbable. It also lacks muscles and most probably is an outgrowth of the second segment of the palpus. In the larval Micropterygid S a b a - t i n c a, however, there is a well-developed three-segmented palpus, a slender, sharply pointed galea, and a broader, rounded lacinia, as described by Tillyard (1922).

The partial fusion of the basal segment of the palpus with the galea in many insects has led many authors to misinterpret the basal segment of the palpus as the palpifer. The basal segment of the palpus has been called the palpifer in many insects by Crampton (1921, 1925, 1928), in larval Panorpa by Steiner (1930), in larval Rhagium by Boving and Craighead (1931), and in larval Noctuidae by Ripley (1924).

The origin of the muscles of the galea upon the stipes is against the view of Crampton (1925) and Hansen (1893, 1930) that the galea is an endite lobe of the 'palpiferal segment'. Snodgrass's view that the lacinia and galea are the subdivisions of the endite of the stipital segment is based on the substantial evidence that the muscles of both lobes arise upon the stipes.

9. THE LABIUM.

The labium of insects is a composite structure formed by the union of two maxilla-like appendages. It has been homologized with the second maxillae of Crustacea, but certain authors regard it as the appendages corresponding to the first maxillipeds of Crustacea. The union of the bases of the first maxillipeds of certain Crustacea and the interpretation of the superlinguae of insects as appendages homologous with the maxillulae (first Maxillae) of Crustacea are the main evidence for the view that the labium of insects corresponds to the first maxillipeds of Crustacea. Crampton (1921), however, homologizes the superlinguae of insects with the paragnaths of Crustacea and the first and second maxillae of insects with the first and second maxillae of Crustacea respectively.

The labium of insects is composed of two segments. The distal segment bearing the palpi and lobes is called the prementum and the proximal segment the postmentum. The suture or flexible area between the prementum and the postmentum is the labial suture. The postmentum in many insects contains two plates with a suture or flexible area between them. The proximal plate is the submentum and the distal the mentum.

There is no doubt that the prementum is the result of union of two stipites of a pair of maxilla-like appendages, hence it is called the labiostipites, but the homology of the postmentum is

still a matter of controversy. Many authors regard it as the united labio-cardines, while certain authors such as MacGillivray (1923) have not even hesitated to compare the submentum and mentum with the proxicardo and disticardo respectively. The detailed discussion will be found in the section dealing with the musculature.

10. The Prementum.

The prementum or labiostipites is formed by the union of two stipites of a pair of maxilla-like appendages. The paired nature of the prementum is suggested by the distal cleft between its stipital components in primitive insects or by the presence of paired sclerites in the ventral wall in certain adult insects, e.g. Pterosticus (Snodgrass, 1935), and also by the origin of the muscles of the palpi and lobes upon it.

The size of the prementum (figs. 22-3, *prm.*, Pl. 3; figs. 24, 26-7, *prm.*, Pl. 4; figs. 28-31, *prm.*, Pl. 5; figs. 32-3, 35-6, *prm.*, Pl. 6; figs. 37-8, 40, *prm.*, Pl. 7; fig. 45, *prm.*, Pl. 9; figs. 61, 62, 64, *prm.*, Pl. 12) varies in different insects and even in the same insects in different stages. In most larval insects it is very small as compared with the postmentum, but in larval Odonata it is larger than the postmentum.

The sclerotization of the ventral part of the prementum forms a single ventral plate in larval insects. The lateral areas bearing the palpi are sometimes demarcated as distinct sclerites, known as the palpigers (fig. 23, *pg.*, Pl. 3) which are counterparts of the palpifers of the maxillae. In certain adult insects the premental sclerotization forms two lateral sclerites, but it is never subdivided into a distal and a proximal plate, the latter bearing the insertion of the 'median muscles'. This has been supposed to be the case in larval Coleoptera and larval adult Neuroptera by Snodgrass (1935), but his 'proximal and distal plates' are really the mentum and prementum respectively and the so-called 'median muscles' inserted upon the 'proximal plate' are the submentomental muscles.

In larval Diptera (figs. 56-60, Pl. 11) the prementum is a strongly sclerotized plate without palpi and lobes. In larval Bibio it is most probably fused with the mentum (figs. 59, 60, *p.*, Pl. 11).

The labial palpus (*lp.*) is shorter than the maxillary palpus and composed of fewer segments. In most larval Coleoptera it is two-segmented and in larval Neuroptera it is three-segmented. Crampton (1921) states that the number is a distinctive characteristic of larval Coleoptera and Neuroptera and maintains that in certain larval Neuroptera such as Chrysopa, the many-segmented condition of the palpus is due to secondary division of the primary three-segmented palpus. However, the larva of Pteronidea has a three-segmented palpus, while larval Mecoptera have again a two-segmented palpus. On the other hand, certain larval Coleoptera such as Ptinus (fig. 35, Pl. 6) have only one segment in the palpus. It is doubtful, therefore, if the number of palpal segments is of fundamental importance.

In larval Lepidoptera and Trichoptera (figs. 45–9, Pl. 9; 50–5, Pl. 10) again, the palpus is one-segmented and is represented by a small lobe on each side of the spinneret. In larval Tipula and Bibio (figs. 56–60, Pl. 11) the labial palpus is absent, unless it is fused with the prementum.

The typical number of terminal lobes borne by the distal part of the prementum is four. The median pair form the glossae, the lateral pair the paraglossae. They are undoubtedly the counterparts of the laciniae and galeae respectively. They vary much in different insects. The median lobes or the pair on each side may be united; again, all lobes may be fused to form a single structure which may be variously modified according to the mode of feeding. There may be a reduction in number, either the glossae and paraglossae being absent, or even all lobes may be totally atrophied.

In larval insects they generally form a single lobe, the ligula (fig. 22, *h.*, Pl. 3). In certain cases (figs. 24–6, Pl. 4; fig. 36, Pl. 6) the pair on each side is fused. In larval Trichoptera, Lepidoptera, and Hymenoptera the terminal lobe bearing the orifice of the labial glands is regarded as the ligula (figs. 48, 49, *h.*, Pl. 9; figs. 50, 52, *sp.*, Pl. 10).

11. The Postmentum.

The postmentum is the basal part of the labium. The sclerotization of the postmentum is extremely variable. In

certain larval insects it forms a single plate as shown by the larvae of Tenebroides and of the Elateridae (figs. 28, 31, *pmt.*, Pl. 5). In the larval sawfly it forms a triangular median plate; in most cases it forms a proximal plate, the submentum (figs. 22, 23, *sm.*, Pl. 3), and a distal plate, the mentum (*m.*). In larval Trichoptera of the families Hydropsychidae, Hydroptilidae, and Philopotamidae (figs. 47–9, Pl. 9) the postmentum has a definite submentum (*sm.*) lying between the two hypostomal areas and a mentum (*m.*), but in the family Limnophilidae (fig. 45, Pl. 9) the postmental sclerotization forms a single proximal plate (*sm.*) which is homologous with the submentum (*sm.*) of figs. 47–9, Pl. 9. Therefore the plate *sm.*, in fig. 45, Pl. 9, is the submentum and the distal membranous area is most probably the mentum (*m.*).

Again, the entire postmentum may be membranous, e.g. in larval Carabidae, Curculionidae, Panorpa, and Tipula (fig. 29, *pmt.*, Pl. 5; fig. 40, *pmt.*, Pl. 7; fig. 57, *pmt.*, Pl. 11). In larval Panorpa and in the Curculionidae the postmental area merges imperceptibly into the basimaxillary membrane and it is rather difficult to determine its outer limit.

It is most interesting to note that the mentum (figs. 37, 38, *m.*, Pl. 7) is sometimes formed of two sclerites, one on each side, each with a submentomental muscle (*22*) inserted upon it. This distinctive feature is exhibited by certain Neuropterous larvae. In the Trichopterous larvae of the family Hydropsychidae (fig. 47, Pl. 9) the mentum (*m.*) is deeply notched distally, the notch extending up to the middle of the plate.

In certain larval insects the postmentum or submentum is partially or wholly adherent to the head capsule between two hypostomal processes (fig. 27, Pl. 4; fig. 37, Pl. 7; figs. 45–9, Pl. 9). They may also be fused with the gula to form a single gulamental plate (figs. 37, 38, Pl. 7; fig. 64, Pl. 12).

12. THE MUSCULATURE OF THE LARVAL LABIUM.

The musculature of the larval labium comprises the following muscles (figs. 22, 23, Pl. 3; figs. 24, 26, 27, Pl. 4; figs. 29, 30, 31, Pl. 5; figs. 33, 35, 36, Pl. 6; figs. 38, 39, Pl. 7; figs. 41,

42, 43, Pl. 8; fig. 46, Pl. 9; figs. 52, 53, 54, 55, Pl. 10; figs. 56, 57, 58, 60, Pl. 11; figs. 61, 63, Pl. 12):

(19) Median muscles of the prementum.—Origin on the post-mentum or submentum, or on the tentorium, insertion ventrally on the middle of the proximal border of the prementum.

(20) Lateral muscles of the prementum.—Same origin, insertion ventrally on the sides of the proximal border of the prementum.

(21) Dorsal muscles of the prementum.—Same origin, insertion on the proximal border of the dorsal side of the prementum.

(22) Submentomental muscles (retractors or flexors of the mentum).—Origin on the posterior region of the sub-mentum, insertion on the proximal border[1] of the mentum.

(23) Retractor of the palpus.—Origin on the prementum, insertion on the base of the palpus. In most larval insects the labial palpus is not provided with antagonistic muscles, but in larval Odonata it has two muscles, a levator and a depressor (Munscheid, 1931).

(24, 25) Dorsal muscles of the silk press. A pair of muscles arising on the dorsal side of the prementum, inserted on the sclerotized raphe of the anterior wall of the press.

(26) Ventral muscle of the silk press.—Origin on the ventro-lateral side of the prementum, insertion on the lateral side of the silk press.

The following additional muscles are found in certain larval insects:

(27) Cranial flexors of the prementum.—Arise on the post-occipital ridge of the cranium, run ventral to the body of the tentorium to be inserted on the dorso-lateral sides of the prementum; present in larval sawflies. Parker (1934) suggests that these muscles should be called the dorsal retractors of the prementum (dorsal

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[1] Except larval Dermestes in which they are inserted in the middle of the mental plate.

muscles), but from their point of origin it seems that the muscles most probably represent the tergal muscles of the labial appendages.

(28, 29) Dorsal and ventral retractors of the spinneret.— Origin on the body of the tentorium, insertion on the dorsal and ventral side of the spinneret; present in larval sawflies.

(30) Transverse muscle of the mentum.—A stout muscle attached to the sides of the mentum; a fine thread runs from the middle of the muscle and is inserted on the dorsal side of the prementum; present in larval Sialis.

(31) A pair of muscles arising on each side of the premental cone, inserted on the hypopharyngeal bracon; present in larval Curculionidae.

Owing to the atrophy of the lobes or their union to form a single small lobe in larval insects, the muscles of the lobes are absent. The typical lobes of the adult insects are described here to show their homologies. The muscles of the glossa and paraglossa (fig. 61, *fgl.*, and *fpgl.*, Pl. 12) in adult insects are almost similar to those of the lacinia and galea, each having a flexor arising upon the prementum, but the muscle corresponding to the cranial flexor of the lacinia is not present in the glossa.

In many larval insects, if it is desired to interpret the homologies of the sclerites, it is absolutely necessary to give a detailed description of the point of origin of the muscles of the prementum and also to describe the submentomental muscles (when present).

In larval Neuroptera, as shown by the larvae of Raphidia and Sialis (fig. 38, Pl. 7; figs. 41, 42, Pl. 8), all muscles of the prementum, i.e. median, lateral, and dorsal muscles (*19, 20, 21*), have their origin on the tentorium. The submentomental muscles (*22*) are also present, having their origin upon the posterior region of the submentum and their insertion on the proximal border of the mentum. In larval Raphidia each submentomental muscle is inserted upon each lateral sclerite of the mentum.

In larval Thanatophilus (figs. 24, 26, Pl. 4), however, the dorsal and lateral muscles (*21, 20*) arise upon the tentorium,

but the median muscles (*19*) of the prementum and also the submentomental muscles (*22*) have their origin on the submentum. The same condition of the premental muscles exists in larval Elateridae (fig. 31, Pl. 5), but the median muscles arise upon the postmentum, there being one plate; consequently the submentomental muscles are absent.

In larval Tenebrio and Ptinus (fig. 23, Pl. 3; fig. 35, Pl. 6), on the other hand, the median and dorsal muscles (*19, 21*) arise from the tentorium, but the lateral muscles (*20*) of the prementum and also the submentomental muscles (*22*) arise from the submentum (*sm.*).

In certain larvae all muscles of the prementum are not retained, either one or two sets being absent. In larval Dermestes (fig. 36, Pl. 6) the median muscles are absent, but the lateral and dorsal muscles (*20, 21*), including the submentomental muscles (*22*), have their origin upon the submentum (*sm.*). In Lucanidae (fig. 30, Pl. 5) the lateral muscles are absent, but the median and dorsal muscles (*19, 21*) of the prementum arise upon the tentorium. The submentomental muscles (*22*) are also retained.

It seems from the point of origin of the premental muscles and submentomental muscles that all of these muscles represent the sternal muscles of the labial appendages, having their primary origin upon the submentum. The shifting of the bases of the premental muscles on to the tentorium is probably rather a secondary condition.

13. The Musculature in Relation to the Sclerites of the Larval Labium.

The insect labium shows so much variation and modification of its sclerites and lobes that it is very difficult in some cases to homologize the sclerites and lobes without examining the musculature.

The prementum can be identified by the origin of the muscles (when present) of the palpi and lobes upon it and also by the insertion of the premental muscles upon its proximal border. The points of insertion of the median, lateral, and dorsal muscles (*19, 20, 21*) limit the proximal border of the prementum

(fig. 22, *prm.*, Pl. 3; fig. 35, *prm.*, Pl. 6; fig. 38, *prm.*, Pl. 7; fig. 41, *prm.*, Pl. 8), and the portion of the labium proximal to the insertion of these muscles is the postmentum. When the postmentum is a single plate (figs. 28, 31, *pmt.*, Pl. 5) no muscle is inserted upon it, but when it has two plates, the submentum and mentum, the latter has the insertion of the submentomental muscles (*22*) upon its proximal border[1] in many larval insects (fig. 22, Pl. 3; figs. 26, 27, Pl. 4; fig. 30, Pl. 5; fig. 35, Pl. 6; fig. 38, Pl. 7; figs. 41, 42, Pl. 8). The mental plate (*m.*), therefore, lies distal to the insertion of the submentomental muscles (*22*) and proximal to the insertion of the premental muscles (*19, 20, 21*). The plate (*sm.*), proximal to the insertion of the submentomental muscles, is evidently the submentum. The origin of the submentomental muscles (*22*) and some of the premental muscles upon the posterior region of the submentum identifies the submental plate. Similarly, the origin of some of the premental muscles (especially the median muscles (*19*)), identifies the postmentum (fig. 31, *pmt.*, Pl. 5) when it is a single plate. Therefore, almost the whole of the postmentum is included between the origin of some of the premental muscles upon the posterior region of the postmentum and their insertion upon the proximal border of the prementum.

The point of origin of the median muscles (*19*) upon the posterior border of the postmentum and their insertion upon the proximal border of the prementum definitely shows that the entire plate (fig. 31, *pmt.*, Pl. 5) in larval Elateridae is the postmentum. Ford (1917) describes the plate, *pmt.*, as the mentum. Roberts (1921) and Crampton (1928) regard this elongated plate as the submentum and the membranous part distal to the plate as the mentum. It is more probable that, as in many other insects, the membranous part is the flexible area between the prementum and postmentum.

In many cases the postmentum has been wrongly called either mentum or submentum, but an examination of the musculature can hardly fail to indicate its true identity.

When the postmentum or submentum is fused with the gula

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[1] Except larval **Dermestes** (fig., 36 Pl. 6) in which the submentomental muscles (*22*) are inserted upon the middle of the mental plate (*m.*).

so as to form a single gulamental plate, it is not difficult to find out the approximate proximal limit of the postmentum or submentum by the points of origin of the submentomental muscles (*22*) or some of the premental muscles (especially the median muscles, (*19*)). The points of origin of these muscles correspond approximately with the pregular suture (when the gula and submentum are separated by a suture) (fig. 22, Pl. 3) or with the line drawn across the two tentorial pits (figs. 37, 38, Pl. 7) which always corresponds with the pregular suture when the latter is present.

Crampton (1921, 1923, 1925, 1928) labels the plate (*sm.*) in larval Hydrophilus (fig. 27, Pl. 4) as the gula and the flexible area distal to it as the submentum, but the origin of the submentomental muscles (*22*) upon the conical proximal border of the plate (*sm*), and their insertion upon the proximal border of the mentum (*m.*), show that the plate (*sm.*) is the submentum with a distal flexible area between it and the mentum.

In larval Raphidia (figs. 37, 38, Pl. 7), where the submentum (*sm.*) is fused with the gula (*gu.*), Crampton (1928) calls the entire plate (*sm.* and *gu.*) the gula and the flexible area distal to the plate the submentum. The point of origin of the submentomental muscles, which also approximately corresponds with the line drawn across the two tentorial pits (*pt.*), shows that the part of the big plate distal to the two tentorial pits is the submentum (*sm.*), and the part proximal to the pits is the gula (*gu.*). The broad plate, labelled *sm.*, in Sialis larva (figs. 41, 42, Pl. 8), is the submentum alone, as revealed by the origin and insertion of the submentomental muscles (*22*). The plate (*sm.*) cannot be a gulamental plate as it is supposed to be by Crampton (1921, 1928).

Snodgrass's view (1935) that the only muscles having their origin upon the postmentum are the median muscles of the labium that extend from the postmentum to the prementum is not upheld in many of the species I have examined. As I have already stated, in addition to submentomental muscles (which are quite different from the median muscles) some of the muscles of the prementum in many larval insects have their origin upon the submentum (fig. 22, Pl. 3; fig. 26, Pl. 4; figs. 35, 36,

Pl. 6). In larval Thanatophilus (fig. 26, Pl. 4) both median and submentomental muscles (19, 22) arise upon the submentum (sm.). In larval Dermestes (fig. 36, Pl. 6) the lateral and dorsal muscles (20, 21), including the submentomental muscles (22), and in larval Tenebrio and Ptinus (fig. 22, Pl. 3; fig. 35, Pl. 6), the lateral and submentomental muscles (20, 22) have their origin upon the submentum (sm.).

Snodgrass (1935) does not distinguish the median muscles from the submentomental muscles and calls the submentomental muscles the 'median muscles' in many larval Coleoptera and larval and adult Neuroptera. This led him to misinterpret the mentum as the 'proximal sclerite of the prementum' and consequently the submentum as the postmentum in such cases. He states that in many larval Coleoptera[1] and larval and adult Neuroptera the prementum is composed of two principal plates, on the proximal one of which are inserted the 'median muscles' and on the distal one the tentorial adductors. A comparative study of the musculature in a wider range of larval insects shows that the so-called 'median muscles' in such cases are the submentomental muscles which are morphologically quite different from the median muscles, the latter being sometimes present at the same time and both having their origin upon the submentum (fig. 26, Pl. 4), and the so-called 'proximal plate' of the prementum is, therefore, really the mentum, and the so-called 'postmentum' is the submentum. In adult Chrysopa (fig. 63, Pl. 12) the plate, labelled m., is the mentum, having the insertion of the submentomental muscles (22) at its proximal border, and the plate, labelled sm., is the submentum. Therefore, the prementum is never subdivided into a proximal and a distal plate, though it may have two lateral sclerites.

In larval Lepidoptera (fig. 50, Pl. 10) the plate (pmt.) is evidently the postmentum as it lies proximal to the insertion of the premental muscles. In many Lepidopterous larvae the postmentum remains membranous like that of the sawfly larva.

In larval Trichoptera (figs. 45, 49, Pl. 9) the postmental

[1] Anderson (1936), supporting Snodgrass's view, states that the prementum in Coleopterous larvae is very often subdivided into a first and a second prementum.

sclerotization forms a single plate in some cases, two in others. No muscles arise from or are inserted upon it, but the entire postmental area lying between the insertion of the premental muscles (*19*) and the two tentorial pits (*pt.*) can easily be recognized. Therefore, the plates (*sm.* and *m.*) in figs. 47-9, Pl. 9, are the submentum and mentum respectively, but in the case where the postmentum forms a single proximal plate (fig. 45, Pl. 9), the plate (*sm.*) undoubtedly represents the submentum which is homologous with the submentum of figs. 47-9, Pl. 9, in shape and position, and the membranous area distal to the plate (*sm.*) is the mentum. In all Trichopterous larvae the submentum (figs. 45-9, Pl. 9) lies either partially or wholly between the two hypostomal processes of the head capsule. Many authors regard the plate (*sm.*) as the gula, but the gula is never situated distal to the tentorial pits.

In larval Diptera (figs. 56-60, Pl. 11) it is rather difficult to interpret the labium with the help of the musculature, since the muscles of the labium arise upon the head capsule. In larval Tipula (figs. 56-8, Pl. 11) the plate (*prm.*) has the insertion of the muscles (*19, 20*) which are homologous with the median and lateral muscles of the prementum, but they arise upon the posterior region of the head. From the insertion of these muscles upon the proximal border of the plate (*prm.*) it can be inferred that the plate represents the prementum, and the membranous area connecting the plate with the hypostoma cannot be anything but the postmentum (fig. 57, *pmt.*, Pl. 11). In Tipulidae Becker (1910) describes the ventral hypostomal plate (figs. 56, 59, *hst.*, Pl. 11) as the submentum and the upper plate (*prm.*) as the mentum. In Chironomus larvae Miall and Hammond (1900) also describe the similar ventral plate as the submentum and the upper plate as the mentum. In larval Bibio (fig. 58, Pl. 11) only the lateral muscles (*20*) are retained, but they are inserted at a point half-way from the proximal border of the plate (*pl.*), suggesting that some element from the postmentum, most probably the mentum, has entered into the composition of the plate (*pl.*); the plate *sm.* is, therefore, the submentum.

The homology of the postmentum is still uncertain. The view that the postmentum contains the sternal element of the labial

segment is strongly supported by the origin of the premental and submentomental muscles upon the postmentum or rather upon the submentum. These muscles represent the sternal muscles of the labial appendages, having their primary origin upon the sternal area of the postmentum. The shifting of the bases of the premental muscles on to the tentorium so as to provide a stronger base for muscles is a secondary condition. In M a c h i l i s Snodgrass states that the median triangular area which is separated by faint lines from the lateral areas of the postmentum is the sternum of the labial segment. This triangular area seems to correspond with the points of origin of the submentomental muscles and the premental muscles upon the postmentum. Imms (1934) suggests that the postmentum, though representing the united cardines, also has a sternal element in its composition.

Crampton (1921, 1928), following the interpretation of Holmgren (1909), regards the entire postmentum as the sternal derivative of the labial segment, and his hypothetical midlabium representing the united cardines lies between the prementum and postmentum. However, from the insertion of the submentomental muscles upon the proximal border of the mentum, it is difficult to justify the inclusion of the mentum in the sternum, for the sternal element must always lie proximal to the insertion of the submentomental muscles. Evidently the mentum is the representative of the united cardines in the labium. The presence of paired sclerites in the mentum[1] of certain Neuropterous larvae is especially suggestive of this theory. If this is the case, then the entire submentum is a sternal derivative. This view seems to agree with the fact that all premental muscles (including submentomental muscles) have their primary origin upon the submentum. The presence of submentomental muscles when there are two plates in the postmentum, and their complete atrophy in a single postmental plate, show that the fusion of the submentum and mentum[2] has taken place

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[1] Compare also incomplete fusion of the two sclerites of the mentum in larval Hydropsychidae (Trichoptera).

[2] It might be objected to this view that many generalized insects have the postmentum represented by a single plate. In certain Orthoptera

late in phylogeny. These facts are scarcely compatible with the inclusion of portions of the labial cardines in the submentum. Therefore, the submentum is entirely a derivative of the sternum of the labial segment, and the only part comparable to the cardines is the mentum. The insertion of the submento-mental muscles representing the sterno-cardinal muscles of the labial appendages on the proximal border of the mentum also strongly supports this interpretation.

A further comparative study of the musculature and sclerites of the labium and embryological data are needed before speculating further.

14. The Musculature of the Mouth-parts of Larval Insects in Relation to Classification.

The musculature of the mouth-parts is very helpful in grouping the larval insects, since each group has its own characteristic muscles, but it is more uniform in some orders, such as Lepidoptera and Trichoptera than in others. On the other hand, in Coleoptera and Neuroptera, so far as I have studied them, each family is in some way peculiar in the musculature of the mouth-parts.

The larvae of Lepidoptera and Trichoptera can at once be distinguished from others in having an additional muscle of the maxillary palpus, which arises upon the cranium and also by the loss of the promotor of the cardo. The cranial muscle of the palpus is characteristic of these two groups and is found nowhere else. The larvae of Trichoptera can be separated from those of Lepidoptera in having both anterior and lateral muscles of the labrum, while in the latter only the lateral muscles are retained. The origin of the cranial muscles of the palpus upon the hypostomal region is again characteristic of larval Lepidoptera, this muscle arising upon the posterior region of the cranium in larval Trichoptera.

The larvae of Mecoptera and Diptera are conspicuous by the absence of all muscles of the cardo. They are also characterized

(e.g. Periplaneta, Gryllus, &c.), however, the postmentum is divided though apparently without muscles I hope to study this point at a later date

by having the dorsal cranial flexor of the stipes (except T i p u l a).
Larval Diptera (Nematocera) can be distinguished by the absence of tentorial adductor and retractor of the stipes and also of the muscles of the maxillary palpus, which are retained in larval Mecoptera. In larval Mecoptera only the anterior labral muscles are present, while in larval Diptera only the lateral labral muscles are retained.

Larvae of Hymenoptera, Neuroptera, and Coleoptera[1] can be distinguished from other larvae so far studied in having the promotor of the cardo. The larvae of Hymenoptera and Neuroptera have both anterior and lateral muscles of the labrum, and larval Coleoptera have either lateral or posterior labral muscles.

The fact that all premental muscles have their origin on the tentorium is characteristic of the larval Neuroptera. The submentomental muscles are not present in any order besides Coleoptera and Neuroptera. Larval Hymenoptera have distinctive dorsal cranial flexors of the prementum. These muscles most probably represent the tergal muscles of the labial appendages.

15. The Gula.

The gula (fig. 22, *gu.*, Pl. 3; figs. 24, 26, *gu.*, Pl. 4; fig. 28, *gu.*, Pl. 5; figs. 37, 38, *gu.*, Pl. 7) is the median ventral plate, formed by the sclerotization of the neck membrane proximal to the postmentum or submentum, and bounded on each side by the extension of the postgenae but demarked from them by the gular suture (figs. 37, 38, *gs.*, Pl. 7). Snodgrass (1928, 1935) considers that the origin of the gula is generally associated with the prognathous condition of insects, in which the head is turned upwards upon the neck, with the result that the original posterior surface becomes the ventral. In such cases the ventral parts of the head become elongated with the extension of the postgenal areas. The membrane on the ventral side of the head behind the postmentum or submentum becomes sclerotized and forms a single median plate, the gula, which gives more rigidity to the head. The membrane forming the gula was originally

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[1] Except larval Elateridae and Trogositidae.

in the neck region behind the postmentum or submentum, but with prognathism has become a part of the head.

That the gula is developed from the cervical membrane is shown by the fact that a pair of muscles (fig. 22, *gum.*, Pl. 3) arising upon the posterior border of prothoracic segment is inserted on a crescent-shaped groove at the anterior margin of the gula, just behind the pregular suture (*pgs.*) in larval T e n e - b r i o . These muscles are homologous with muscles having the same origin but inserted on the proximal border of the membrane just behind the submentum in certain larval insects, where the gular area is not sclerotized.

Comstock and Kochi (1902) consider that the gula is the sternum of the cervical segment which has migrated cephalad. They cite C o r y d a l i s as their example and state that the sternum of the cervical segment forms the gula but the sternellum remains behind it. Stickney (1923) contradicts them, stating that both sternum and sternellum of the 'cervical segment' are retained behind the gula in C o r y d a l i s . Henriksen (1928) maintains that the sternum of Dermaptera forms the gula, but that in beetles the gula is a quite different thing and has nothing to do with the sternum. Stickney (1923) defines the gula as the sclerite formed by the migration of the posterior tentorial pits from the occipital foramen towards the submentum and emphasizes that it must be derived from the postgenae. From the condition exhibited by the larvae of T e n e b r i o we can scarcely derive the gula from the postgenae. Besides, there are many insects (e.g. larval Carabidae) where the tentorial pits lie far forward from the occiput, yet the gula is not developed.

The presence of a gula is not strictly characteristic of prognathous insects, since it is also found in certain hypognathous types in a reduced condition. On the other hand, in certain prognathous insects, such as the larva of S i a l i s , the gula is not developed at all, i.e. the area behind the submentum remains membranous. A well-developed gula (fig. 29, *gu.*, Pl. 5; figs. 37, 38, *gu.*, Pl. 7) is found in typical prognathous insects, such as the larvae of R a p h i d i a and T e n e b r o i d e s .

The gula is primarily demarked from the submentum by a

suture, the pregular suture (fig. 22, *pgs.*, Pl. 3). In certain insects the gula is fused with the postmentum or submentum, so as to form a single gulamental plate (fig. 37, *sm.* and *gu.*, Pl. 7; fig. 64, *pmt.* and *gu.*, Pl. 12). A line drawn across the two tentorial pits (fig. 37, *pt.*, Pl. 7) can be taken as defining the anterior limit of the gula in the gulamental plate.

In many insects the two tentorial pits are much elongated and run towards the submentum from near the occipital foramen. The anterior ends of these two elongated tentorial pits (fig. 22, *pt.*, Pl. 3) correspond exactly with the pregular suture in the larvae of Tenebrio and Tenebroides in which the gula is distinctly separated from the submentum and postmentum respectively. Therefore, the gula can also be defined as the plate lying between the two tentorial pits when the latter are elongated.

The points of origin of the submentomental muscles (*22*) or median muscles (*19*) are very useful in defining the gular limit in larval insects (fig. 26, Pl. 4). The submentomental muscles (*22*) arise upon the posterior region of the submentum, slightly in front of its proximal border or pregular suture, in larval Tenebrio (fig. 22, Pl. 3). In larval Raphidia (fig. 38, Pl. 7), where the gula is fused with the submentum, the point of origin of the submentomental muscles (*22*) approximately corresponds with the line drawn across the two tentorial pits (*pt.'*). Although it is not possible to define in this way the exact anterior limit of the gula in the single gulamental plate, one can at least rest assured of its not being extended beyond the point of origin of these muscles. Crampton (1921, 1928) wrongly interprets the gulamental plate as the gula alone in larval Raphidia.

In the soldier termite (Termopsis sp.) the gula (fig. 64, *gu.*, Pl. 12) lies between the two elongated pits (*pt.*), but it is fused with the postmentum (*pmt.*) so as to form a big plate. The portion of the gulamental plate distal to the elongated pits is evidently the postmentum (*pmt.*). Snodgrass (1935) designates the entire plate (*pmt.* and *gu.*) as the postmentum, although he states that 'in some of the Scarabaeidae the gula is likewise demarked by the greatly elongated tentorial pits'. The point

of origin of the median muscles[1] also approximately corresponds with the anterior ends of the tentorial pits.

In certain insects the hypostomal lobes of the head lie in front of the two tentorial pits and the postgenal lobes behind them; and the basal part of the labium becomes fixed to the head capsule in front of the two tentorial pits. The membrane behind the two tentorial pits may form a gula or it may be completely obliterated by the median approximation of the postgenal areas, leaving only a suture, the mid-genal suture. The characteristic modification of the former condition is exhibited by the larva of Raphidia (fig. 37, Pl. 7), in which the submentum (sm.) lies between the hypostomal lobes of the cranium but in front of the two tentorial pits, while the gula (gu.) lies behind them. The second type of modification is shown by larval Hydrophilus (fig. 27, Pl. 4). The submentum (sm.) is similarly fixed to the head capsule but the gular area is obliterated by the median approximation of the postgenal areas, leaving only the mid-genal suture (mgs.). This median approximation has altered the shape of the submentum into a cone which lies slightly behind the two tentorial pits, but the origin and insertion of the submentomental muscles (22) may be relied on in the interpretation of the submentum.

A further modification of the median approximation of both hypostomal and postgenal areas is shown by the larvae of Panorpa, of the Carabidae, and of the Elateridae, in which there is left only a suture (figs. 29, 31, mgs., Pl. 5) between the approximated ventral parts of the head capsule.

In larval Cerambycidae the median approximation has gone a step farther than that exhibited by the larva of Panorpa, &c., resulting in the complete fusion of the ventral parts of the head, which thus forms a bridge, the hypostomal bridge (fig. 23, hst.b., Pl. 3), sometimes wrongly called the gula. However, the tentorial pits lie at the posterior margin of the head capsule and consequently the gula is absent.

In Trichopterous larvae the tentorial pits (fig. 46, pt.', Pl. 9) lie at the posterior margin of the head capsule and the gula is

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[1] The median muscles and submentomental muscles have the same point of origin upon the submentum.

totally absent. The so-called gula in larval Trichoptera is really the submentum (figs. 45, 46, 47, 48, 49, *sm.*, Pl. 9) which lies between the two hypostomal lobes but varies much in shape and relative position. Siltala (1907) maintains that in certain Trichopterous larvae of the families Hydroptilidae and Hydropsychidae the so-called gula is the submentum, and in other families there are two parts of which the posterior one is the gula, but Belton (1934) could not find a suture and states that there is a thickened ridge in the middle of the plate and a distinction in colour. I have also examined a few types of certain families and thoroughly agree with Belton.

Crampton's attempt (1921, 1928) to define the gula by drawing a line between the bases of the maxillary cardines is responsible for many of his misinterpretations of the sclerites of the labium, although he states that the point of origin of the submento-mental muscles is very helpful in defining the gula and submentum.

Crampton's view (1921, 1928) regarding the formation of the gula and submentum in the adult by the secondary division of the primary gulamental plate of the larva is not supported by any substantial evidence. The best example brought forward by him is that the single gulamental plate of the larva of S i a l i s forms the gula and submentum in the adult by secondary division. I have already shown that the plate (figs. 41, 42, *sm.*, Pl. 8) which is called the gulamentum by Crampton is the submentum alone, as revealed by the origin and insertion of submentomental muscles (*22*). In adult S i a l i s, if there is any gula at all (I did not study adult S i a l i s) it must have been secondarily formed by the sclerotization of the membrane behind the submentum of the larva. Secondly, the presence of single gulamental plate in a larval Pyrochroid and a distinct gula and submentum in the adult, though the dividing line between them is obliterated (i.e. in effect a single gulamental plate in the adult also), does not prove that the presence of gulamentum as a single plate is a primitive condition, since in certain beetle larvae (e.g. T e n e b r i o) the gula and submentum are distinct but in the adults they are fused to form a single plate. Again, in certain larvae there is no gula at all, but in their adults a well-developed

gula is formed. Thirdly, the supposed presence of a gula as a part of the broad plate, labelled *mu.*, *sub.*, and *gu.*, in the termite embryo (vide Crampton's fig. 12, 1928) is evidently due to misinterpretation.

Walker (1932) states that the prognathous insects are more primitive than the hypognathous type, but the latter may revert to prognathism with a change of habits. The absence of a gula in hypognathous insects, which retain the primitive ventral position of the mouth appendages, is undoubtedly a generalized condition, since in typical prognathous insects having a well-developed gula the mouth appendages are directed forward. The fusion of the gula with the basal sclerite of the labium is a secondary condition.

16. SUMMARY.

The principal points brought out in the foregoing discussion may be summarized as follows:

1. The points of origin of the anterior, lateral, and posterior labral muscle identify the frons in larval insects.

2. The hypopharynx is proximal to the insertion of the dorsal muscles (when present) of the prementum.

3. The direction of the cardo varies with the prognathous and hypognathous conditions of the insect head.

4. The palpifer is a secondarily demarcated portion of the stipes (Borner and Snodgrass), and cannot be a definite segment of the mouth-part limb, since no muscles take their origin from or are inserted upon it.

5. The lacinia can be recognized by the stipital and cranial flexors, and the galea by the stipital flexor only.

6. The cranial flexor of the lacinia is always retained and is a very important muscle in identifying the single maxillary lobe when one of the two is absent.

7. The origin of the flexors of the lacinia and galea upon the stipes shows that the lobes belong to the stipes.

8. The basal segment of the maxillary palp is often misinterpreted as the palpifer owing to its partial or complete fusion with the basal part of the galea, but it can be definitely identified by the insertion of the palpal muscles upon its base.

9. The premental sclerotization may form two lateral sclerites, but it is never subdivided into a proximal and a distal sclerite.

10. The mentum represents the united cardines of a pair of maxilla-like appendages.

11. The submentum is a derivative of the sternum of the labial segment.

12. The fusion of the submentum and mentum to form a single postmental sclerite is a secondary condition.

13. The sternal muscles of the labial appendages are represented by the premental muscles having their primary origin upon the submentum, and also by the submentomental muscles.

14. With the prognathous condition of the insect head the gula is developed from the cervical membrane.

15. The prognathous insects having a well-developed gula are more specialized than the hypognathous insects in which the gula is either reduced or absent.

16. The larval insects could be classified by the musculature of the mouth-parts, since each group has its characteristic musculature.

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EXPLANATION OF PLATES 1–12.

ABBREVIATIONS.

a., antenna; *adfr*, adfrons; *b*, basal segment of the palpus; *bm.*, basimaxillary membrane; *c.*, condyle; *cl*, clypeus; *dc*, disticardo, *eph.*, epipharynx, *el.*, endite; *fgl.*, flexor of the glossa; *fpgl.*, flexor of the paraglossa; *fr.*, frons; *frcl.*, frontoclypeus; *g.*, ginglymus; *ga.*, galea; *ge.*, gena; *gl*, glossa, *gs*, gular suture; *gu.*, gula; *gum.*, gular muscles; *hcl*, suspensoria; *hph.*, hypopharynx; *hst.*, hypostoma; *hst.b*, hypostomal bridge; *in.*, incisor area; *l.*, accessory lobe, *lc.*, lacinia; *li.*, ligula; *lm.*, labrum, *lp*, labial palpus; *md.*, mandible; *mdc.*, mandibular cavity; *mgs.*, midgenal suture; *mo.*, molar area; *mp.*, maxillary palpus; *mpm*, maxillary papal muscles; *msc.*, basimaxillary sclerite; *p.*, cardo process; *pc.*, proxicardo, *pcl.*, postclypeus; *pf*, palpifer; *pg.*, palpiger; *pgl.*, paraglossa; *pgu.*, paragula; *pl*, plate formed by the fusion of the mentum and prementum; *pmt.*, postmentum; *pr.*, prostheca; *prm.*, prementum; *pt.*, posterior tentorial pit, *pt.'*, position of the posterior tentorial pit; *sld.*, salivary duct; *sm.*, submentum; *st.*, stipes; *t*, torma; *ta.*, tentorial arm; *tb.*, tentorial bridge; *2*, anterior labral muscles; *3*, lateral labral muscles, *4*, posterior labral muscles; *5*, hypopharyngeal muscles, *6*, abductor muscle of the mandible, *7*, adductor muscle of the mandible; *8*, promotor of the cardo; *9*, adductors of the cardo; *10*, adductors of the stipes, *11*, retractor of the stipes; *12*, cranial flexor of the lacinia; *13*, stipital flexor of the lacinia; *14*, stipital flexor of the galea; *15*, levator of the palpus; *16*,

depressor of the palpus; *17*, cranial flexor of the stipes; *18*, cranial flexor of the maxillary palpus; *19*, median muscles of the prementum; *20*, lateral muscles of the prementum; *21*, dorsal muscles of the prementum; *22*, submentomental muscles; *23*, retractor of the palpus; *24* and *25*, dorsal muscles of the silk press; *26*, ventral muscles of the silk press; *29*, dorsal and ventral retractor of the spinneret; *30*, transverse muscle of the mentum.

PLATE 1.

Figs. 1–11.—Labrum showing its muscles.

Fig 1.—Larva of S i a l i s   l u t a r i a .

Fig. 2.—Larva of C a u l o p h i l u s   l a t i n a s u s .

Fig. 3 —Larva of A n a b o l i a   n e r v o s a .

Fig. 4.—Larva of C a r a b u s   s p

Fig. 5.—Larva of D e r m e s t e s   v u l p i n u s .

Fig. 6.—Larva of T e n e b r o i d e s   m a u r i t a n i c u s .

Fig. 7.—Larva of P a n o r p a   c o m m u n i s .

Fig. 8.—Larva of A g r i o t i s   sp.

PLATE 2.

Fig. 9.—Larva of P t e r o n i d e a   r i b e s i i .

Fig. 10.—Larva of T h a n a t o p h i l u s   sp.

Fig. 11 —Larva of R a p h i d i a   sp.

Fig 12.—Larva of C a l a n d r a   g r a n a r i a . Left mandible (anterior view) showing its muscles

Fig. 13.—Larva of L u c a n u s   c e r v u s . Left mandible showing its molar area.

Fig. 14.—Larva of L u c a n u s   c e r v u s . Mandible (dorsal view).

Fig. 15.—Larva of C a r a b u s   sp. Right mandible (posterior view).

Fig 16.—Larva of C h r y s o p a   sp. Left mandible (posterior view).

Fig. 17 —Larva of A n a b o l i a   n e r v o s a . Right mandible (posterior view) showing its muscles.

Fig. 18.—Larva of T i p u l a   f l a v o l i n e a t a . Right mandible (posterior view) showing its muscles.

Fig 19.—Larva of S i a l i s   l u t a r i a . Left mandible (posterior view).

PLATE 3.

Figs. 20, 21.—Larva of T e n e b r i o   m o l i t o r . Right maxilla (dorsal view) showing some of its muscles.

Fig. 22.—Larva of T e n e b r i o   m o l i t o r . Labium and gula (dorsal view) showing their muscles

Fig 23 —Larva of R h a g i u m   sp   Maxillae and labium (dorsal view) showing their muscles.

PLATE 4.

Fig. 24 —Larva of T h a n a t o p h i l u s   sp. Maxilla and labium (dorsal view) showing some of their muscles.

Fig 25.—Larva of Thanatophilus sp. Maxilla (dorsal view) showing some of its muscles

Fig. 26.—Larva of Thanatophilus sp. Labium (dorsal view) showing some of its muscles.

Fig. 27.—Larva of Hydrophilus piceus. Maxilla and labium (dorsal view) showing their muscles.

PLATE 5.

Fig. 28.—Larva of Tenebroides mauritanicus. Maxilla with labium (dorsal view) showing some of its muscles.

Fig. 29 —Larva of Carabus sp Maxillae and labium (dorsal view) showing their muscles.

Fig. 30.—Larva of Sinodendron cylindricum. Maxilla and labium (dorsal view) showing their muscles.

Fig. 31.—Larva of Agriotes sp. Maxilla and labium (dorsal view) showing their muscles.

PLATE 6

Fig. 32.—Larva of Calandra granaria. Maxillae and labium (ventral view).

Fig. 33.—Larva of Calandra granaria. Labium (dorsal view) showing its muscles.

Fig. 34.—Larva of Ptinus tectus Maxilla (dorsal view) showing some of its muscles.

Fig. 35.—Larva of Ptinus tectus. Labium (dorsal view) showing its muscles

Fig. 36.—Larva of Dermestes vulpinus. Labium (dorsal view) showing its muscles.

PLATE 7.

Fig. 37 —Larva of Raphidia sp. Ventral view of the head capsule with maxilla and labium.

Fig. 38.—Larva of Raphidia sp. Labium with gula (dorsal view) showing its muscles.

Fig. 39.—Larva of Raphidia sp. Distal part of the maxilla (dorsal view) showing its muscles.

Fig. 40.—Larva of Panorpa communis. Maxillae and labium (dorsal view) showing their muscles.

PLATE 8.

Fig 41 —Larva of Sialis lutaria. Maxillae and labium (dorsal view) showing their muscles.

Fig 42.—Larva of Sialis lutaria. Labium (dorsal view) showing some of its muscles.

Fig. 43.—Larva of Pteronidea ribesii. Labium (dorsal view) showing some of its muscles.

Fig. 44.—Larva of Pteronidea ribesii. Maxilla (dorsal view) showing some of its muscles.

PLATE 9.

Fig. 45.—Larva of Anabolia nervosa Ventral view of the head capsule with maxillae and labium

Fig. 46.—Larva of Anabolia nervosa. Maxilla and labium (dorsal view) showing their muscles.

Fig. 47.—Larva of Hydropsyche sp. Labium (ventral view).

Fig. 48.—Larva of Philopotamus sp. Labium (ventral view).

Fig. 49 —Larva of Hydroptila sp. Labium (ventral view).

PLATE 10.

Fig. 50.—Larva of Galleria mellonella Maxillae, labium, and hypostoma (ventral view).

Fig. 51.—Larva of Galleria mellonella. Distal part of the maxilla (ventral view).

Fig. 52 —Larva of Galleria mellonella. Muscles of the prementum labial glands (dorsally dissected).

Fig. 53.—Larva of Galleria mellonella. Muscles of the labial glands (ventral view).

Fig 54.—Larva of Galleria mellonella. Maxilla (dorsal view) showing palpal muscles

Fig. 55.—Larva of Galleria mellonella. Maxilla and prementum (dorsal view) showing their muscles

PLATE 11

Fig. 56 —Larva of Tipula flavolineata. Maxilla and labium (dorsal view) showing their muscles.

Fig. 57.—Larva of Tipula flavolineata. Labium (lateral view) showing its muscles.

Fig. 58.—Larva of Tipula flavolineata. Prementum (ventral view) showing its muscles.

Fig. 59.—Larva of Bibio sp. Maxilla and labium (ventral view).

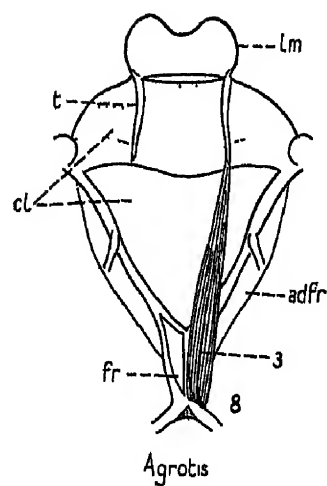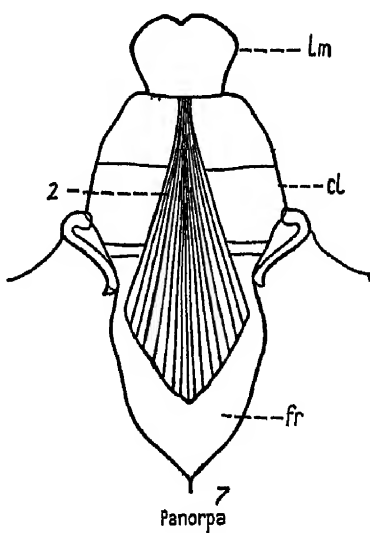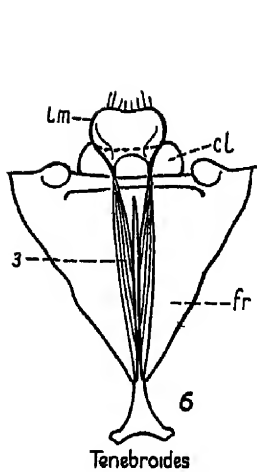Fig 60.—Larva of Bibio sp. Maxilla and labium (dorsal view) showing their muscles.

PLATE 12.

Fig. 61.—Adult of Periplaneta americana. Labium (dorsal view) showing its muscles.

Fig. 62.—Adult of Periplaneta americana. Maxilla (dorsal view) showing some of its muscles.

Fig. 63 —Adult of Chrysopa sp Labium (dorsal view) showing its muscles.

Fig 64.—Adult of Termopsis (soldier). Labium with gula (ventral view).

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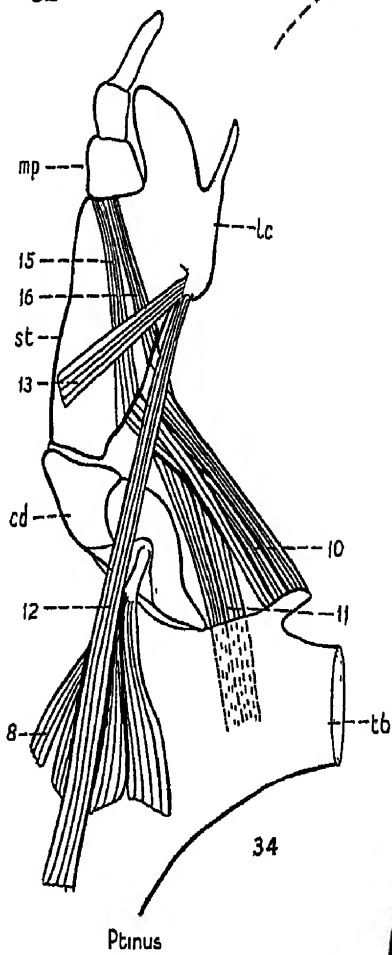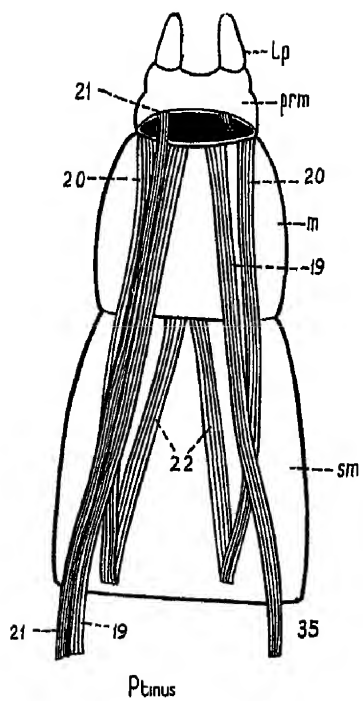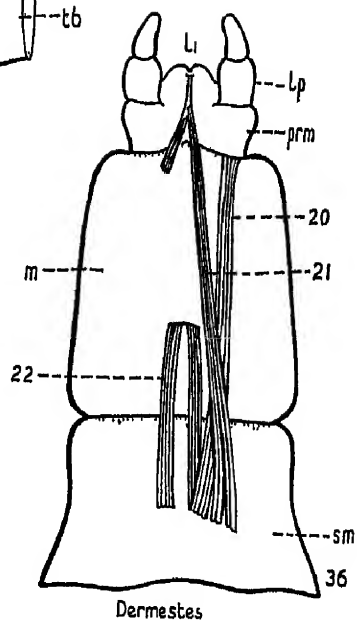
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Periplaneta

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Chrysopa

Periplaneta   62

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Termopsis
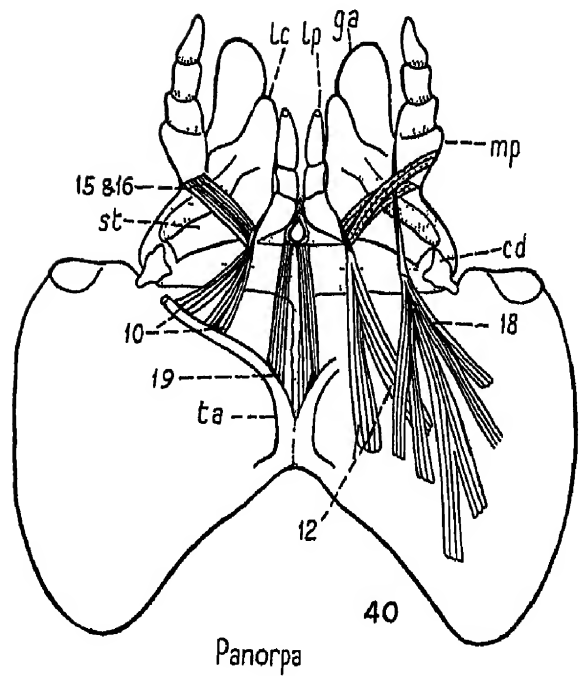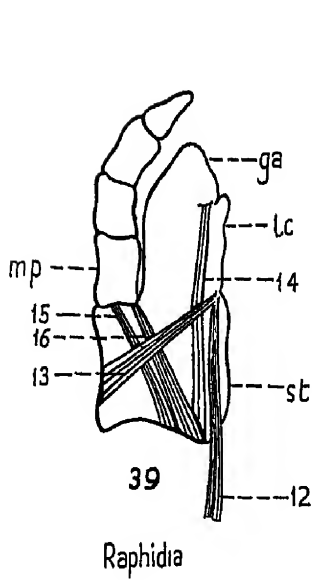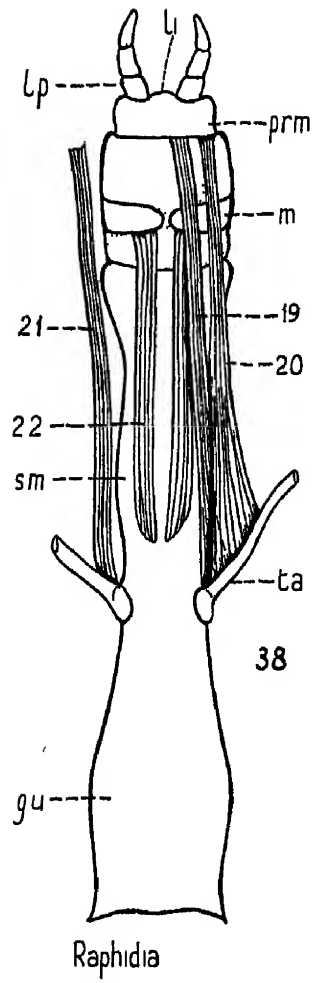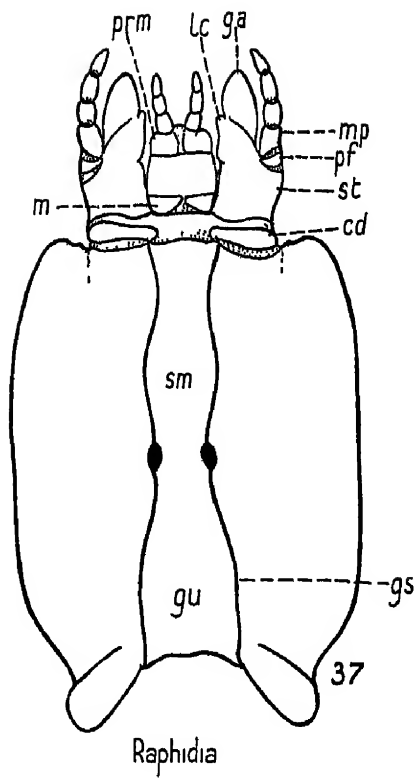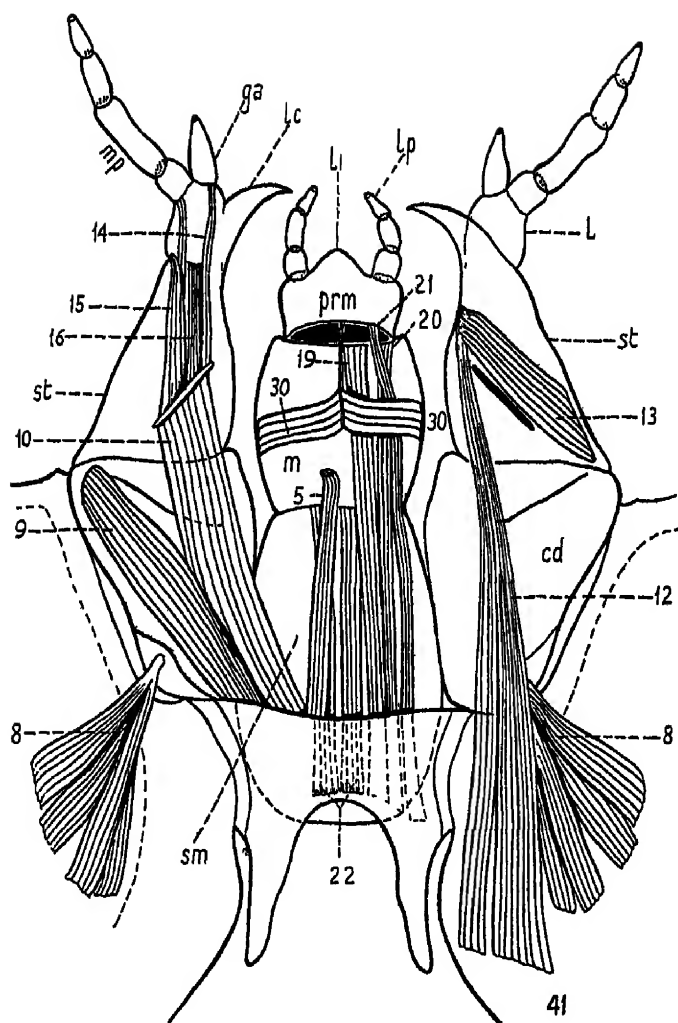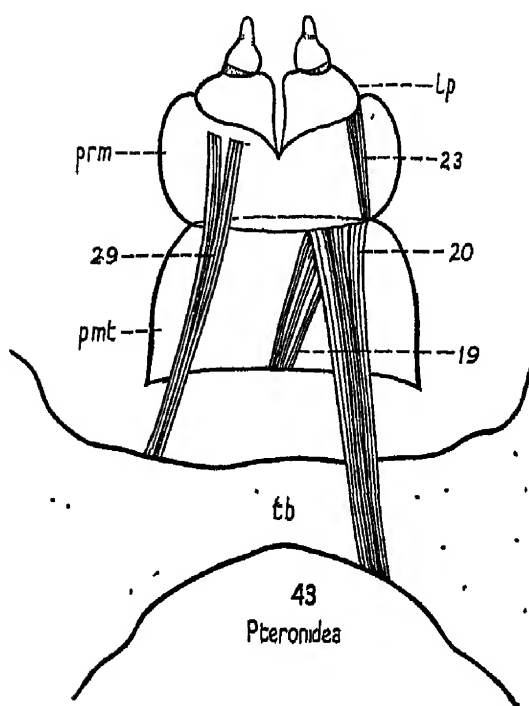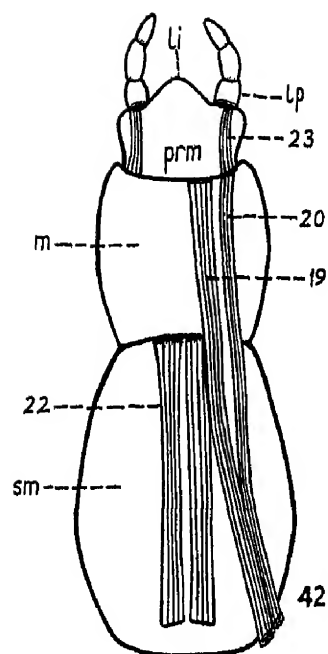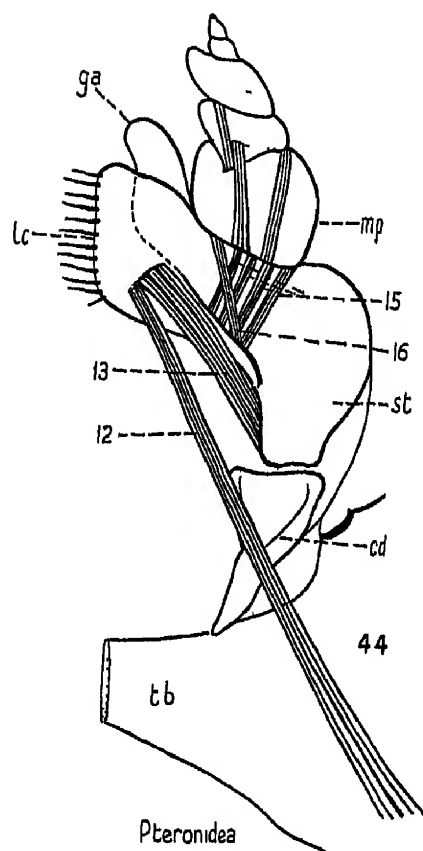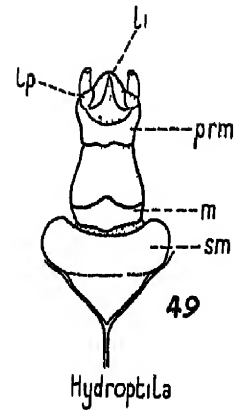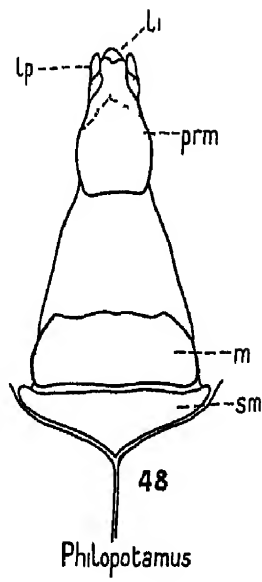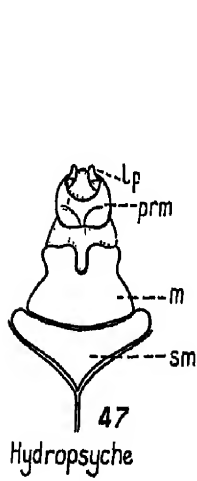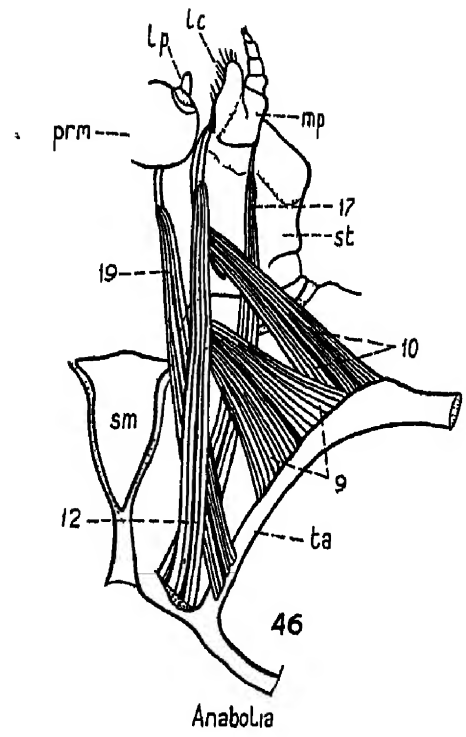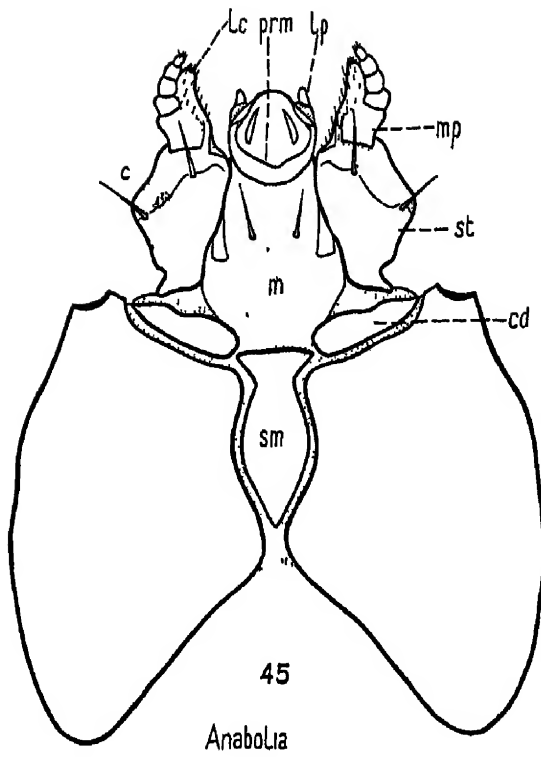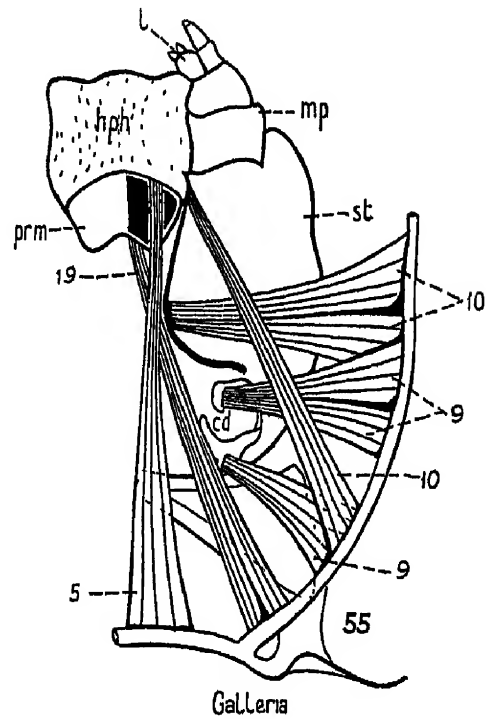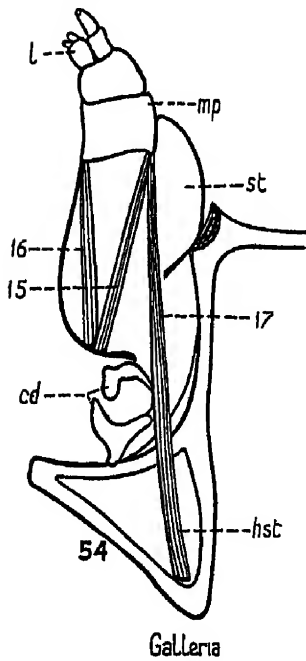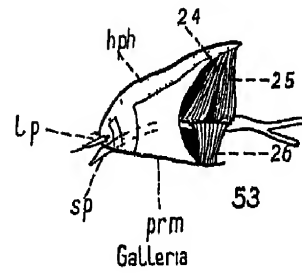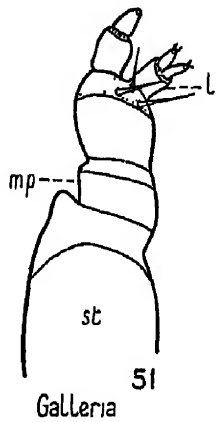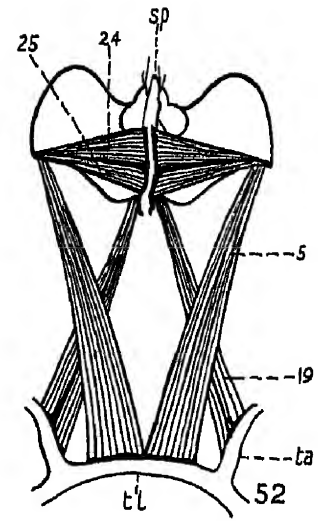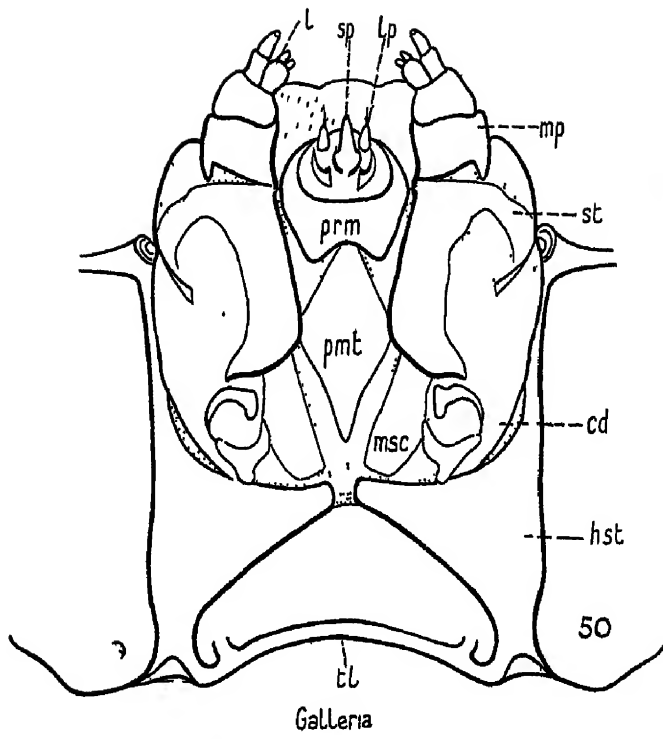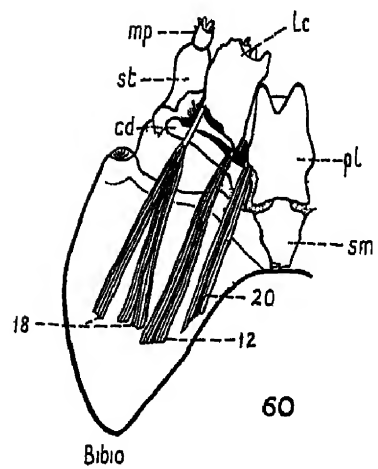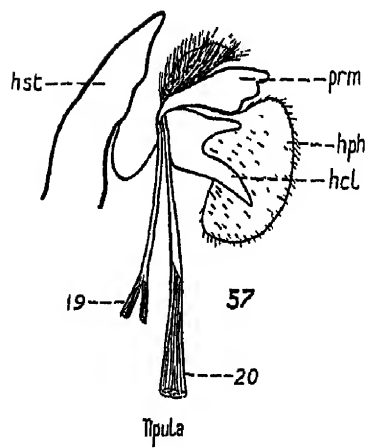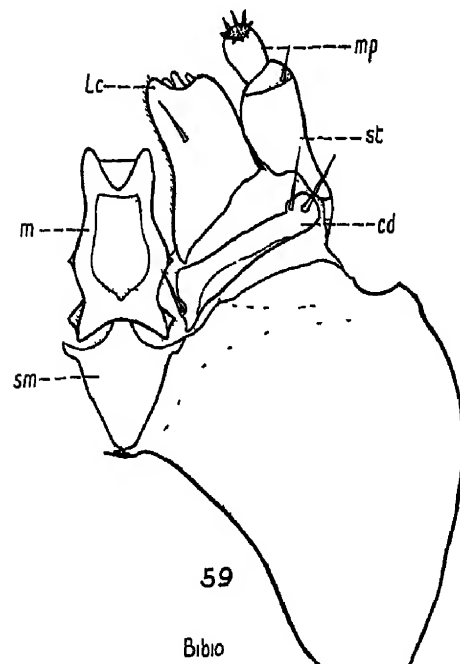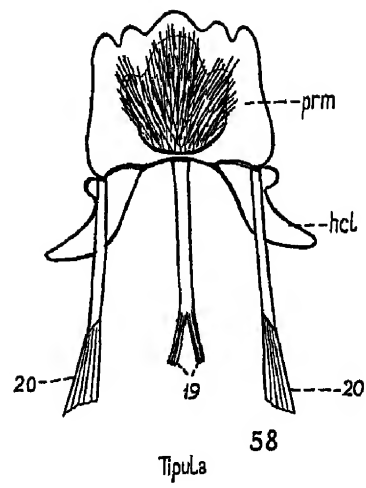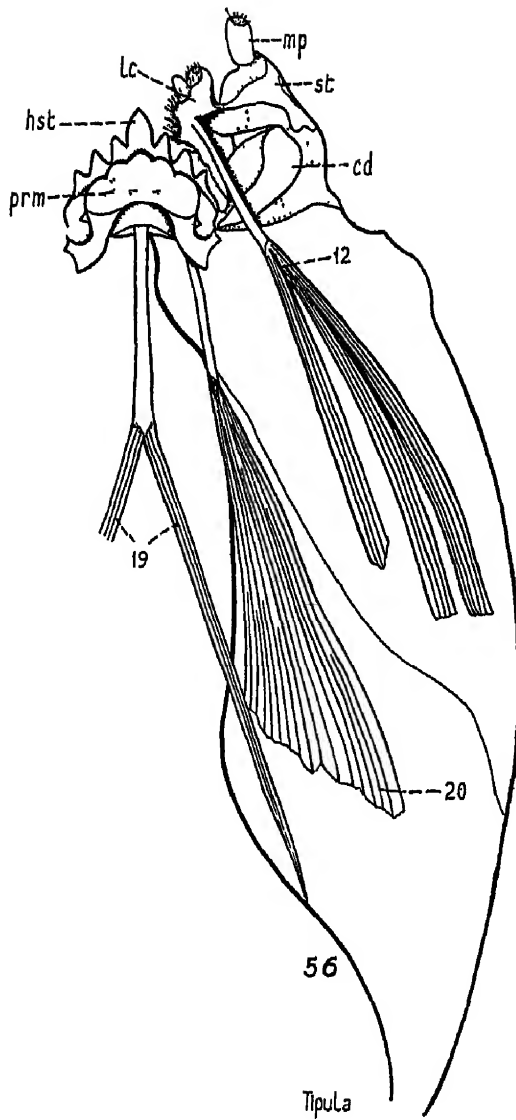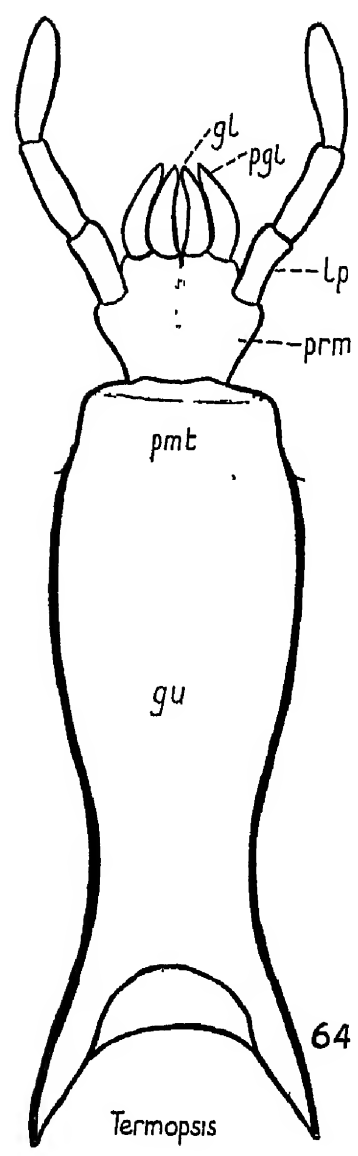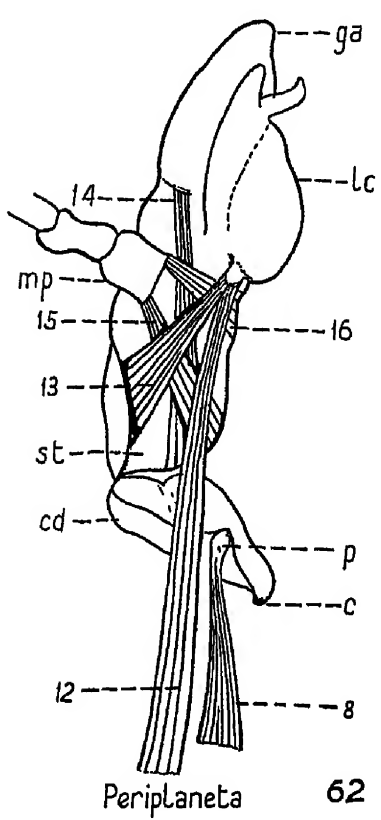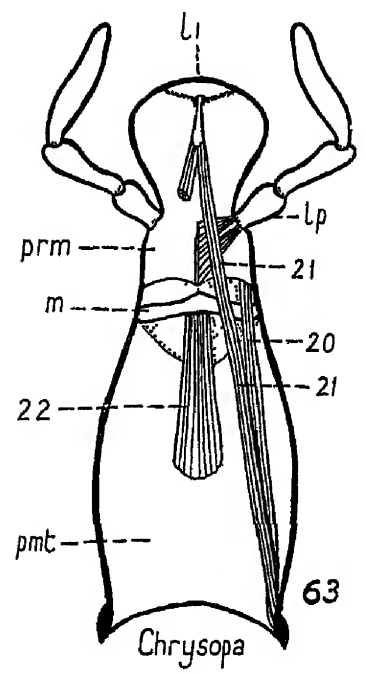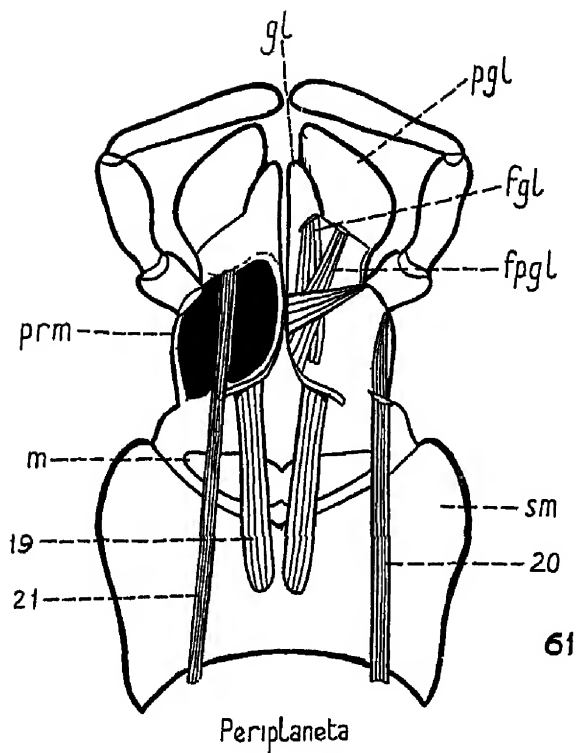
The Development of the Adrenal Gland of the Cat.

By

Sarah Davies, M.Sc.,

University of Liverpool.

With Plate 13.

CONTENTS.

I. INTRODUCTION.

THIS paper deals with the development of the adrenal gland of the cat. A search of the literature has revealed that very little work has been done on this subject.

In 1903 Soulié described the development of the adrenal gland of the cat during foetal life. He states that the first appearance of the adrenal is seen in a foetus measuring between 6 and 7 mm. in length, where it is represented by small centres of cell proliferation in the coelomic epithelium. At the 12 mm. stage it is represented by a homogeneous mass of cells adhering to the wall of the inferior vena cava. In a foetus of 16 mm. the cells of this mass have assumed a reticular arrangement. Between the 16 mm. and 18 mm. stages the rudiment (anlage) of the gland is invaded by parasympathetic elements derived from near-by sympathetic abdominal ganglia. These parasympathetic elements continue to penetrate the gland until the 4 cm. stage, after which very little further invasion is seen The parasympathetic cells pass to the centre of the gland and give rise

to the medulla, whilst the original rudiment forms the cortex of the gland. He says that in a foetus of 9 cm. the cortex is fully differentiated into an outer zona glomerulosa, a middle zona fasciculata, and an inner zona reticularis. He further states that at birth the gland has practically assumed the adult structure, although there is still a slight intermingling of cortical and medullary tissue at the junction of the two regions. The zona fasciculata is said to be very narrow at this stage while the zona reticularis, on the other hand, is very extensive. This latter observation is interesting in the light of subsequent investigations described below.

A considerable amount of work has been done on the development of the adrenal in other mammals.

In 1911 Elliott and Armour recorded that in the early human foetus the adrenal cortex consists of an outer narrow zone of small cells and an inner highly vascular mass of cells free from fatty substances and forming the greater part of the foetal cortex. At this stage the medulla is said to be represented by small groups of cells scattered here and there throughout the inner mass. The large size of the human suprarenal gland during foetal life is stated to be due to a marked hypertrophy of the inner cortical mass, which begins at a very early stage and continues until birth. At full term the medulla is said to be entirely central. Immediately after birth the inner cortical mass begins to degenerate and has completely disappeared by the end of the first year. Meanwhile, at the end of the seventh month, the cells of the outer narrow zone assume the appearance and arrangement of the cells of the adult cortex[1] and store up fatty substances. These cells alone develop into the adult cortex.

In 1912 Glynn recorded that the human adrenal during foetal life is at first larger than the kidney, this large size being due to a hypertrophy of the inner portion of the foetal cortex. This begins to degenerate immediately after birth, and the adult cortex is developed from an outer rim of small cells.

In 1919 Jackson recorded that in the newly born albino rat

[1] Elliott and Armour give no details in regard to the different layers of the cortex.

the zona glomerulosa, zona fasciculata, and zona reticularis are all present in the cortex, lipoids being uniformly distributed throughout all three zones. He states that at birth cortex and medulla are not distinctly separated, there being a certain amount of intermingling of cells at the junction of the two regions. He further states that during the confluence of the medulla in the first week after birth the cortical cell-strands within it are absorbed, and that during further growth the cortex increases rapidly, the medulla but slowly.

In 1922 Weymann recorded that in the pig embryo the medulla is formed by cell groups, derived from the sympathetic ganglia, penetrating the cortical anlage and accumulating in the centre of the gland.

The first really detailed account of the intra-uterine development of any adrenal was given in 1925 by Cooper, who worked on the human foetus. According to Cooper's description, in a foetus of 8 weeks the gland consists of an outer narrow zone surrounding a central mass. The outer zone consists of small cells with vacuolate cytoplasm and deeply staining nuclei. The central mass consists of large, angular cells loosely arranged and separated by intercellular spaces occupied by capillaries. These cells are granular and possess large nuclei with deeply staining nucleoli and nuclear membrane. (At 3 months these cells are described as eosinophil.) 'The peripheral cells are epithelial in origin and form the cortical anlage of the developing suprarenal gland, while the central cells, derived from the cortical anlage, constitute the boundary zone' (loc. cit., p. 34). No further details are given of the origin of the boundary zone. At a later stage, during the third month of intra-uterine life, the gland still consists of an outer narrow zone surrounding a central mass. The outer zone still consists of small cells with deeply staining nuclei and vacuolate cytoplasm, and the central mass consists of large cells lying end to end in a reticular manner and enclosing capillary spaces. These cells present an almost syncytial appearance, their boundaries being difficult to distinguish. Their cytoplasm is granular, and they possess large pale nuclei, with deeply staining nucleoli. 'The narrow zone of small deeply stained cells at the periphery of the adrenal body is the true

cortex. The large central mass is the boundary zone and is also part of the foetal cortex' (loc. cit., p. 36). At the junction of cortex and boundary zone, however, lying free in the spaces between the cells of the latter, are small masses of cells with intensely vacuolate cytoplasm and very deeply staining nuclei. According to Cooper these are ectodermal sympatho-chromaffin cells which have made their way into the adrenal gland from accumulations of such cells situated between the adrenal bodies and the kidneys. These accumulations represent the future abdominal sympathetic ganglia. As development proceeds the outer zone or true cortex continues to increase in size, its cells becoming more vacuolated and arranged in columns like those of the fasciculate layer of the adult cortex. The boundary zone diminishes in size, while the number of sympatho-chromaffin cells increases, and they become more concentrated in the centre of the gland. At mid-term most of these cells are in the centre of the gland, but a few are still scattered in the boundary zone. After mid-term all have reached the centre of the gland. During the eighth month, and at the conclusion of intra-uterine life, the cortex is wide and is differentiated into two zones, an outer narrow zona glomerulosa, immediately within the capsule surrounding the gland, and an inner wide zona fasciculata. The cells of both zones are vacuolate, those of the zona glomerulosa being arranged in small groups and those of the fasciculate zone in columns. 'The inner ends of the columns are penetrating into the boundary zone and forming the zona reticularis' (loc. cit., p. 39). The boundary zone has diminished in size and the centre of the gland is occupied by masses of sympatho-chromaffin cells, many of which are developing into medullary cells with granular cytoplasm, whilst others are developing into sympathetic ganglion cells. The latter, however, were not found in all cases. At this stage there is no true medulla.

During early post-natal development the cortex increases in size, the boundary zone gradually disappears, and the medulla becomes fully defined, consisting of loosely arranged cells with granular cytoplasm and large pale nuclei. With the complete disappearance of the boundary zone at about 18 months the cortex comes into direct contact with the medulla. At this stage

the zona reticularis is still ill defined. At 3 years, when the gland assumes the structure characteristic of the adult, the zona reticularis is well marked and consists of 'large, finely vacuolated cells in reticular arrangement, enclosing large blood spaces in the meshes' (loc. cit., p. 42).

Since my own research on the adrenal of the cat, herein recorded, another worker in this department has investigated the development of the mouse adrenal in great detail (Waring, 1935). He has shown that in the mouse the cortical anlage arises at about the twelfth day of foetal life from the mesenchyme. During the fourteenth day this cortical mass is invaded by sympatho-chromaffin cells which gradually migrate to the centre of the gland and form the medulla. The outer region of the cortical anlage gradually becomes differentiated to form the adult cortex, the glomerular, fasciculate, and reticular layers appearing in order from the periphery inwards, but there is no previous distinction between an outer zone and an inner mass such as is reported for the human gland. A large part of the original cortical mass, in the mouse, takes no part in the formation of the adult cortex and remains as a broad zone of relatively undifferentiated tissue lying between the cortex and the medulla, and interlocking with the medulla. This 'interlocking zone' remains till about the thirtieth day after birth in the male, until a considerably later period in the virgin female, or until the first pregnancy. Waring points out that this interlocking zone is probably homologous with the boundary zone of man, but that the homology is not certain because the origin of the boundary zone is not known.

Thus, according to Souhé (1903), Weymann (1922), Cooper (1925), and Waring (1935), the medulla is described as arising from cells of sympathetic origin, the sympatho-chromaffin cells, which invade the cortical anlage and accumulate in the centre of the gland. According to all investigators of the human adrenal—Elliott and Armour (1911), Glynn (1912), and Cooper (1925)—the foetal cortex consists of an inner and outer portion, the latter alone giving rise to the adult cortex, while the inner part degenerates during development. According to Waring in the mouse also the whole adult cortex arises by differentiation

of the outer part only of the foetal cortex, the inner part degenerating though not till adult life. According to Soulié (1903), however, in the cat this inner portion of the foetal cortex becomes the reticular zone of the adult cortex.

My own investigation has shown that, contrary to the statement of Soulié (1903), the development of the adrenal gland in the cat shows the same sequence of events as that described in man.

II. Material.

The material used for the present investigation came to hand incidentally in the course of a study of variations in the adrenal gland of the cat. As the material was not collected systematically for the present research, it is in many respects very limited.

For the purpose of the larger investigation 111 animals were dissected. Of these 18 were sub-adults and 18 were pregnant females. As all the animals were obtained from the general population, neither age nor stage of pregnancy was known.

In the case of the pregnant females the foetuses were removed and measured laterally[1] from the crown of the head to the anus, these measurements being taken as a very rough indication of the stages of development. Naturally, such measurements are not a reliable indication of the exact age of the embryos. The difficulty of measuring makes the margin of error comparatively large; also the individual variation in size of the foetuses from one animal is considerable, and it is almost certain that the variation in foetuses of the same age from different animals is even greater. Nevertheless, the lengths of the foetuses do give some indication of their stage of development.

All the foetuses were removed from every pregnant female obtained, but in most cases it has only been possible to examine the glands of one foetus from each litter. In one instance every member of a litter of five (48 hours post-natal) was examined,

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[1] The foetuses were measured l a t e r a l l y owing to a misunderstanding. Foetuses are usually measured along the m i d - d o r s a l l i n e from the crown of the head to the root of the tail. My measurements, therefore, though perfectly valid for comparison with each other, are not comparable with those of other workers.

and all the adrenals were found to be at the same stage of development.

The sub-adults were weighed in order to obtain some indication of their age. Clearly such weights are no real criterion—they serve only to separate the very young from those distinctly older. Consequently, their glands, as described below, are not necessarily arranged in exact order of age; but the general order of adrenal development is clear.

III. Technique.

All the cats were chloroformed, and in every case both glands were removed while the body was still warm. All adherent extraneous tissue was dissected away and one gland of each pair was fixed in Bouin's fluid, the other in Zenker's fluid modified after Helly, the former proving the more suitable for histological work. The glands were sectioned at a thickness of 6μ and stained in Ehrlich's haematoxylin and eosin.

IV. The Adrenal Gland of the Cat at Different Stages of Development.

Descriptions are given below of the adrenals of eighteen foetal and eighteen sub-adult glands.

A. Foetal Glands.

The glands are described in what appears to be the order of their adrenal development.

Stage No. 1. (See fig. 1, Pl. 13.) (Two foetuses, from different mothers, were examined at this stage of adrenal development, each measuring 3 cm. in length.)

A transverse section of the glands of these foetuses showed a central mass of cells surrounded by a single narrow outer zone. This outer zone consisted of compactly arranged small cells with pale granular cytoplasm and pale, well-defined nuclei, the nucleoli and nuclear membrane alone being deeply stained. Scattered among these cells could be seen a few large cells with vacuolate cytoplasm and small, black, circular nuclei. The central mass occupied practically the whole of the gland and

consisted of large cells loosely arranged in a reticular manner. The boundaries of these cells were difficult to distinguish. Their cytoplasm was densely granular and strongly eosinophil, and their nuclei were large and well defined, the nucleoplasm being pale in marked contrast to the deeply staining nucleoli and nuclear membrane At the periphery of the central mass a few of the large vacuolate cells with small black nuclei were seen lying freely among the granular cells, but they were entirely absent from the more central regions of the gland.

S t a g e N o . 2. (Three foetuses were examined, all from different mothers. Two measured 5 cm. in length and one measured 5·5 cm)

This stage presented the same general appearance as Stage 1, but the cytoplasm of the cells of the peripheral zone was slightly vacuolate. Also the large vacuolate cells with small deeply stained nuclei were more numerous in the periphery of the central mass than in Stage 1 described above, and many such cells could now be seen also in the centre of the gland whither the other cells of this type appeared to be migrating.

S t a g e N o . 3. (See fig. 2, Pl. 13.) (One foetus was examined; length 7 cm.)

The general appearance was somewhat the same as in Stage 2, but the cells of the central mass, where it abutted on to the outer zone, tended to be more compactly arranged than those nearer to the centre of the gland. Both the peripheral narrow zone and the outer region of the central mass were comparatively free from the vacuolate cells which were now more concentrated in the centre. Apparently the process of immigration, the beginning of which was seen in the glands previously described, is here more advanced.

S t a g e N o . 4. (One foetus was examined; length 8 cm.)

The cells of the narrow zone at the periphery of the gland here tended to be arranged in small groups, separated by connective tissue continuous with the outer capsule. Internal to this zone was a wider zone of large, compactly arranged, deeply granular cells evidently belonging to the original central mass,

the remainder of which formed a loose reticulum in the centre. The large, intensely vacuolate cells with small black nuclei were but sparsely scattered throughout the peripheral zone and compact outer region of the central mass, and were more concentrated in the reticular portion occupying the centre of the gland.

Stage No. 5. (One foetus of this stage was examined and measured 9 cm. in length.)

The gland presented very much the same picture as that of Stage 4, but now the large, intensely vacuolate cells occupied the greater part of the centre and were almost confined to that region. Some strands of the original central mass were still present in the central region.

Stage No. 6. (One foetus was examined; length 9 cm.)

The peripheral zone now showed an increase in width as compared with previous stages. The vacuolate type of cell was even more predominant in the centre of this gland than hitherto, the densely granular, deeply staining cells of the original central mass tending to be relegated to a zone lying immediately within the original peripheral zone.

In some sections a large group of a totally different type of cell was visible at the periphery of the central mass adjacent to the outermost zone. These cells possessed granular cytoplasm, in which they resembled the granular type of cell of the central region, but their nuclei were similar to those of the vacuolate type of cell, being small, circular, and deeply stained. These cells were not found in any other gland and they are not at present understood.

Stage No. 7. (One foetus examined; length 10 cm.)

The general appearance was very similar to that of the preceding stage. The deeply staining cells of the inner wide zone were more compactly arranged where they abutted on to the outer zone, but towards the centre of the gland were still loosely arranged in a reticular manner. The groups of large vacuolate cells in the centre were lying freely in the interspaces of the reticulum.

Stage No. 8. (One foetus was examined; length 10 cm.)

The accumulation of the vacuolate type of cells in the centre of the gland was even more marked than previously.

Stage No. 9. (One foetus was examined, length 10 cm.)

The original peripheral zone of small vacuolate cells was now wider and showed a marked arrangement into two separate regions. The outer, adjacent to the capsule, consisted of small groups of cells partially separated by thin strands of connective tissue. The inner consisted of cells arranged in short radial columns, which at their outer ends were either regularly rounded off or ended against the groups of cells of the outer region. This arrangement of the cells of the original peripheral zone appears to foreshadow the development of the glomerular and fasciculate zones of the adult cortex. Internally the columns merged into the inner wide zone of densely granular, deeply staining cells belonging to the original central mass. A few large, intensely vacuolate cells with small black nuclei were still to be seen scattered among the cells of both the peripheral zone and of the inner wide zone. Practically the whole of the central region of the gland was occupied by cells of the vacuolate type, some of which, however, now possessed granular cytoplasm but still retained the small black nucleus characteristic of the vacuolate condition of the cell.

Stage No. 10. (Two foetuses from different mothers were examined, each measuring 11·5 cm. in length.)

The general appearance remained unchanged. In one gland, however, the continuity of the peripheral zone was occasionally interrupted by strings of the large vacuolate type of cell with small black nuclei, extending towards the centre. A few such cells were still seen in both glands scattered sparsely throughout this peripheral zone, and also throughout the inner wider zone of densely granular, deeply staining eosinophil cells.

Stage No. 11. (Two foetuses from different mothers were examined at this stage of adrenal development, one measuring 12·5 cm. in length and the other 13 cm.)

In the glands of this stage the peripheral zone had increased

considerably in width. Also a larger number of cells of the vacuolate type in the centre of the gland now possessed granular cytoplasm, whilst still retaining the small black nucleus.

S t a g e   N o .   1 2 . (See fig. 3, Pl. 13.) (Two foetuses from different mothers were examined, each measuring 13 cm. in length. They were apparently full-term.)

The general appearance was very similar to that of Stage 11, but the inner zone of densely granular, deeply staining, eosinophil cells had decreased considerably in width. Some of the cells in the central region still possessed vacuolate cytoplasm and small black nuclei, but the majority now had granular cytoplasm, and whilst some of these still retained the small black nuclei characteristic of earlier stages, in others the nuclei had become large and palely staining. These latter cells, in spite of their granular cytoplasm and large nuclei, were clearly distinct from the cells of the original central mass, the latter being much more eosinophil, as in all previous stages of development.

Thus the development of the adrenal gland of the cat during foetal life can be summarized as follows:

In the earliest stage observed the adrenal anlage consists of a central mass of large, strongly eosinophil cells, surrounded by a narrow peripheral zone where the cells are smaller and less deeply staining. Cells of a third type, clearly distinguishable by their highly vacuolate cytoplasm and small black nuclei, are seen scattered in the peripheral region of the gland.

As development proceeds the highly vacuolate cells gradually concentrate in the centre of the gland, whilst the original deeply eosinophil central mass is thereby being relegated to a broad zone surrounding the mass of vacuolate cells and interlocking with it.

The outermost narrow zone of cells can be identified with a similar zone, described by Elliott and Armour (1911), Glynn (1912), and Cooper (1925), in the adrenal of the early human foetus, where it is claimed to be the forerunner of the permanent cortex. The large, strongly eosinophil cells obviously constitute what Elliott and Armour (1911) and Glynn (1912) call the inner portion of the f o e t a l cortex, and what Cooper (1925) terms the boundary zone. They will be referred to henceforth in this

paper as the 'boundary zone'. The large vacuolate cells with small black nuclei are evidently equivalent to Cooper's sympatho-chromaffin cells, and will be referred to in the present paper as sympatho-chromaffin cells. Unfortunately, no foetus was obtained at a sufficiently early stage of development to show the site of origin of the different types of cells observed.

By the end of foetal development the majority of the sympatho-chromaffin cells have been concentrated in the centre of the gland, but still some remain scattered in the boundary zone (see fig. 3, Pl. 13). Some of those in the centre are beginning to show the characters of the typical adult medullary cells. The outermost narrow zone has become differentiated into two zones suggestive of the glomerular and fasciculate zones of the adult cortex, whilst the boundary zone remains unchanged.

After birth further changes take place in the adrenal whereby it gradually assumes the adult condition. Thirteen stages of post-natal development are described below from sub-adult animals. They are arranged in what appears to be the order of development of the adrenal gland.

B. Sub-adult Glands.

Stage No. 1. (One sub-adult only, a female, was obtained at this stage; weight ¼ lb.)

A transverse section of this gland (see fig. 4, Pl. 13) showed an outer wide permanent cortex and an inner wide boundary zone interlocking with a central medulla.

The cortex consisted of two zones only, an outer narrow zona glomerulosa at the periphery of the cortex and immediately within the fibrous capsule, and an inner wide zona fasciculata. The permanent cortex of this gland, and that of all glands subsequently described, was devoid of sympatho-chromaffin cells. The zona glomerulosa in section consisted of small cells arranged in groups separated by connective tissue; the cytoplasm of the cells was vacuolate, and the nuclei were pale and well defined. The zona fasciculata consisted of similar cells arranged in short columns separated by vascular connective tissue septa. The inner ends of the columns penetrated the boundary zone, but no zona reticularis could be identified.

The boundary zone consisted of large, strongly eosinophil cells loosely arranged in a reticular manner and without distinct cell outlines. Towards the periphery of the zone the cells were more compactly arranged, but on the medullary side they were freely intermingled with the medullary cells and formed large trabeculae. The cells of the boundary zone possessed densely granular, strongly eosinophil cytoplasm and large, pale, well-defined nuclei, only the nucleoli and nuclear membrane staining deeply. Lying freely among the granular cells of the boundary zone were seen a few large, vacuolate sympatho-chromaffin cells with small black nuclei.

The medulla consisted of a mass of cells arranged in small groups. Some of the cells were still of the typical sympatho-chromaffin type with vacuolate cytoplasm and small black nuclei. A larger number, however, possessed granular cytoplasm, and of these the majority still had small black nuclei, but others possessed large pale nuclei.

S t a g e N o . 2 . (Five sub-adults were examined, two females and three males, all from the same litter, 48 hours old. Unfortunately they were not weighed. They were all found to be at the same stage of adrenal development.)

The general appearance was the same as in Stage 1, but the boundary zone was a little narrower. Also, a larger number of the sympatho-chromaffin cells were undergoing transformation into typical medullary cells.

S t a g e N o . 3 . (One sub-adult female was examined at this stage; weight ¼ lb.)

This gland was similar to that of the preceding stage, but now by far the larger number of medullary cells possessed granular cytoplasm, some still retaining the small black nucleus characteristic of earlier stages whilst others had the large pale nuclei of the typical medullary cells.

Some typical sympatho-chromaffin cells were still visible scattered freely among the granular cells of the medulla.

S t a g e N o . 4 . (One sub-adult female was examined at this stage; weight ¾ lb.)

The general appearance was essentially the same as in pre-

ceding stages, but the cells at the bases of the columns of the zona fasciculata, facing the centre of the gland, were loosely arranged and intermingled with the cells of the boundary zone. They were, however, clearly distinguishable from the latter, being smaller in size and having cytoplasm which was more vacuolate and less eosinophil than that of the boundary zone.

A few sympatho-chromaffin cells were still seen scattered freely among the medullary cells.

S t a g e N o. 5. (Two sub-adult males were examined at this stage of development; each weighed ¾ lb.)

The general appearance was as in Stage 4, but the cells at the inner ends of the columns of the zona fasciculata were now somewhat granular and more loosely arranged than previously, the whole appearance suggesting the beginning of a reticular zone, but no definite zona reticularis was as yet evident.

The boundary zone, in section, was of variable width, being wide in some places whilst in others it was so narrow as to be hardly distinguishable.

A few typical sympatho-chromaffin cells were still evident among the medullary cells.

S t a g e N o. 6. (One sub-adult female was examined at this stage; weight ¾ lb.)

This gland presented the same essential histological picture as that of the preceding stage, but the medulla was now permeated by large irregular spaces, often containing blood capillaries.

S t a g e N o. 7. (One sub-adult female was examined at this stage. Its weight was not taken.)

The boundary zone was narrower and more ill defined than in any preceding stage; moreover, it did not form large trabeculae in the medulla as in the glands previously described. A certain amount of interlocking of tissue, however, still occurred at the immediate junction of medulla and boundary zone, small groups of medullary cells lying in the intercellular spaces of the boundary zone.

Again only a few unaltered sympatho-chromaffin cells were to be seen lying freely among the medullary cells.

Stage No. 8. (One sub-adult female was examined at this stage; weight ¼ lb)

As in the preceding stage a transverse section (see fig. 5, Pl. 13) showed an outer wide cortex, and an inner narrow boundary zone, ill defined in places, but still interlocking with a central medulla.

The majority of the cells of the zona fasciculata still possessed the pale, round nuclei characteristic of this zone in the younger stages, but in some the nuclei were now small, black, and of irregular outline, characteristic of the cells of the fasciculate layer in the adult. The cells at the bases of the columns had the same appearance as the remainder of the cells of this layer, but were loosely arranged in a definite reticulum and formed what appeared to be a very narrow, ill-defined zona reticularis between the fasciculate layer of the cortex and the remaining boundary zone.

A few vacuolate sympatho-chromaffin cells were still seen in the medullary mass.

Stage No. 9. (One sub-adult male was examined at this stage. Its weight was not taken.)

A transverse section (see fig. 6, Pl. 13) showed that the boundary zone had completely disappeared. The cortex was therefore now in direct contact with the medulla, but there was no interlocking between the two. Three cortical zones were clearly distinguishable, an outermost, narrow but well-defined, vacuolate zona glomerulosa; a middle wide zona fasciculata also vacuolate; and an innermost, narrow but clearly marked zona reticularis consisting of loosely arranged granular cells. That this is the zona reticularis and not the boundary zone is beyond question. The cells here were smaller than those of the boundary zone, had more definite cell walls, and were very much less eosinophil. They correspond exactly with the cells found at the bases of the columns of the fasciculate zone in Stage 8, where they were identified as of the reticular zone type. At that stage there was no possibility of confusing them with the cells of the boundary zone, for both regions were present together, adjacent to each other, and showed up in strong contrast to each other.

The medulla in section was extensive in area compared with the width of the cortex, and a few typical sympatho-chromaffin cells were still visible lying freely among the medullary cells.

S t a g e   N o .   1 0.   (One sub-adult male was examined at this stage, weight $2\frac{1}{4}$ lb.)

The general appearance was as in the preceding stage, but now a larger number of the cells of the zona fasciculata possessed the small, black, irregularly stellate nuclei characteristic of this zone in the adult. Also, the zona reticularis, though still narrow compared with the zona fasciculata, was relatively wider than in Stage 9.

S t a g e   N o .   1 1.   (One sub-adult male was examined at this stage; weight 2 lb.)

The cells of the zona fasciculata were more highly vacuolate, and those of the zona reticularis more densely granular, than in the preceding stage. Further, the cortex in section occupied a relatively larger proportion of the gland, when compared with the medulla, than in the previous stage.

A few unmodified sympatho-chromaffin cells could still be seen intermingling freely with the more typical medullary cells.

S t a g e   N o .   1 2.   (One sub-adult female was examined at this stage; weight $1\frac{3}{4}$ lb.)

The zona reticularis was relatively wider than in the preceding stage, and only a very few, rather small, unmodified sympatho-chromaffin cells could be seen among the medullary cells.

S t a g e   N o .   1 3.   (One sub-adult male was examined at this stage; weight 2 lb.)

The zona reticularis was now relatively much wider. Indeed, the cortex now occupied a much greater proportion of the width of the gland, in section, than in previous stages. No sympatho-chromaffin cells were seen among the medullary cells.

Thus, during the post-natal development of the adrenal gland in the cat, the cells of the boundary zone are gradually relegated to the periphery of the medulla and finally disappear; conse-

quently the cortex and medulla come into direct contact with each other. Before the boundary zone has entirely vanished the reticular zone of the adult cortex makes its appearance, apparently as a differentiation of the inner ends of the fasciculate layer. It is at first narrow and ill defined, but gradually assumes the form of a definite reticular zone. The cortex then increases rapidly in width, owing largely to the greater development of this zone.

With regard to the medulla, its post-natal development consists of the disappearance of the trabeculae of the boundary zone, and the transformation of all the sympatho-chromaffin cells into typical medullary cells.

V. GENERAL DISCUSSION AND SUMMARY.

The observations herein recorded go far to establish the homology of the inner zone of the foetal cortical tissue in the cat with the boundary zone of man. In the cat this zone disappears in early post-natal life, as it does in man, and, contrary to Soulié's claim (1903), it does not give rise to the reticular zone of the permanent cortex, this latter zone apparently arising by differentiation of the inner ends of the columns of cells of the fasciculate zone.

Whether or not the boundary zone in man and in the cat is homologous with the interlocking zone in the mouse is not certain. The three tissues have the same relationship with other parts of the gland in all cases, and in all cases have the same fate of ultimate degeneration. But whereas in the mouse the interlocking zone is known to be the inner part of the original cortical mass (i.e. of the original adrenal anlage before any sympatho-chromaffin cells have invaded it), in man and in the cat the origin of the boundary zone is unknown. In both the latter cases the earliest known stage of development already shows a specialized outer zone surrounding a central mass of cells, the former giving rise to the adult cortex and the latter being the boundary zone.

I wish to express my thanks to Mrs. Bisbee for the help and criticism she has given me throughout this investigation.

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Explanation of Plate.

Abbreviations.

b.z., cells of boundary zone; *f.c.*, fibrous capsule; *m.*, medullary cells; *p z.*, peripheral zone; *s.c.*, sympatho-chromaffin cells; *z.f*, cells of zona fasciculata; *z.g*, cells of zona glomerulosa; *z.r.*, cells of zona reticularis

All the figures represent transverse sections of cat adrenals taken through approximately the middle of the gland. Bouin's fluid and Ehrlich's haematoxylin and eosin.

Plate 13.

Fig. 1.—Adrenal gland of a foetus measuring 3 cm. in length. × 250.

Fig. 2.—Gland of a foetus measuring 7 cm. in length. × 215.

Fig 3.—Gland of a foetus measuring 13 cm. in length, apparently full term. × 255.

Fig. 4.—Gland of a very young female sub-adult at Stage 1 of post-natal adrenal development. × 215.

Fig. 5.—Gland of a female sub-adult at Stage 8 of post-natal adrenal development. × 215.

Fig 6.—Gland of a male sub-adult at Stage 9 of post-natal adrenal development. × 225.
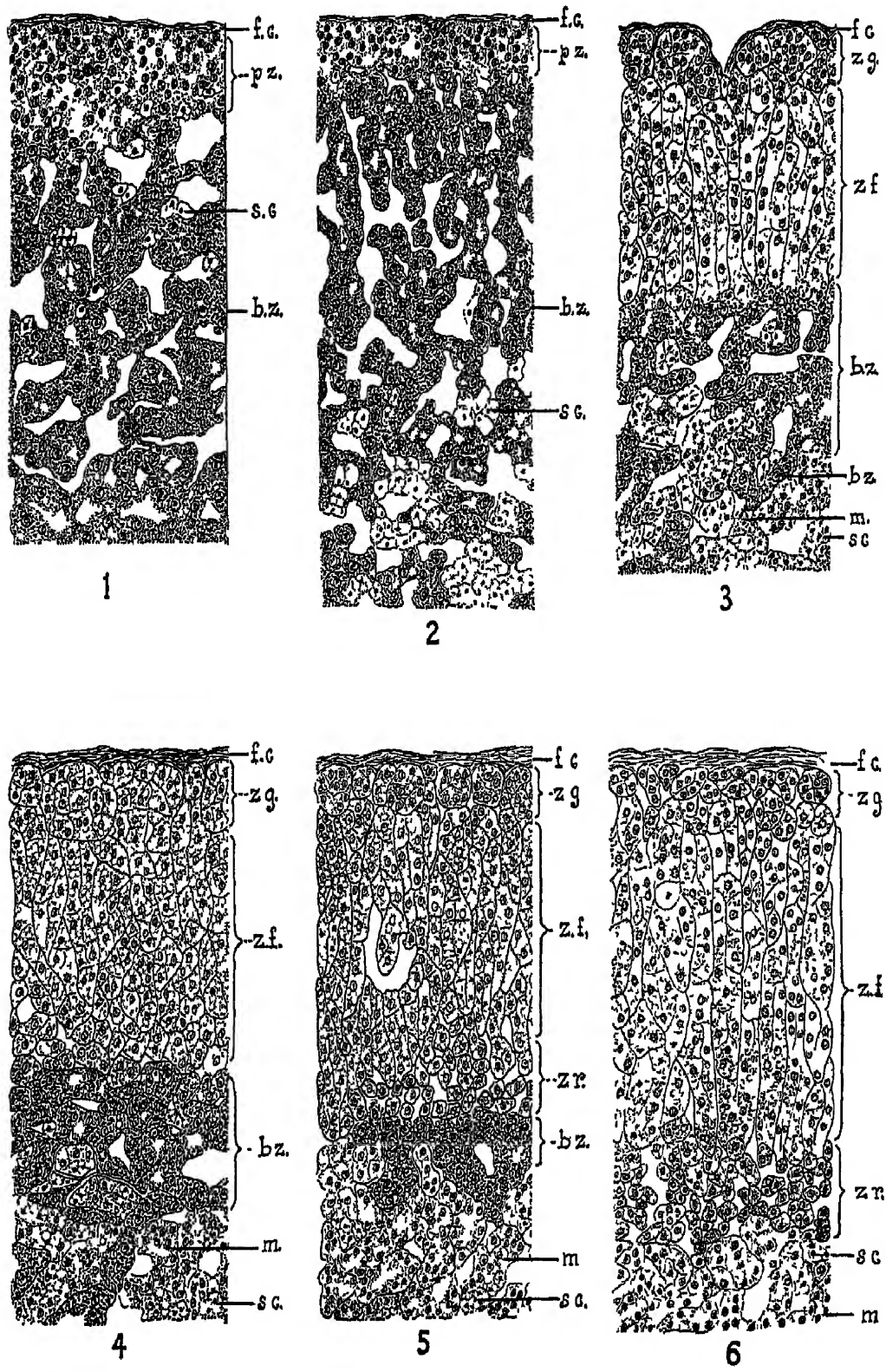
The Reproductive System of the Planarian Artioposthia triangulata (Dendy).

By

Marion L. Fyfe, M.Sc.,

Lecturer in Zoology, University of Otago, New Zealand.

With Plates 14–16 and 3 Text-figures

CONTENTS.

INTRODUCTION.

ALTHOUGH Dendy has described for New Zealand a number of species of land planarians which he allotted to the genus Geoplana yet in all his and Moseley's work on these worms no study has been made of the microscopic internal anatomy, mainly because the classification was based on external features.

In examining specimens of Geoplana triangulata Dendy in serial sections I found that they possess muscular gland-organs or adenodactyli (von Graff) which are characteristic of the genus Artioposthia, no representative of which small group has previously been identified as inhabiting New

Zealand. Other unusual features noted were a branching vas deferens and elongated ovaries situated far back and not in the region of the brain as is usual. Moreover, each ovary is a complex organ containing more than one true Germarium associated with specialized nutritive cells.

I wish to express my thanks to Professor Benham, F.R.S., for originally suggesting the work and for his help and criticism throughout the course of the investigation.

PREVIOUS WORK.

The first record of the occurrence of planarians in New Zealand was made by Hutton (7, p. 249) in 1873 in a paper on the geographical relations of the New Zealand fauna. He merely remarked on the occurrence of two or three species of land planarians, 'one or two of which belong to the genus Bipalium'. Nothing further was added to this until 1877 when Moseley (13) published a detailed description of Geoplana traversii, a small worm measuring about 3 cm. in length. Two mature specimens of this worm had been presented to Moseley by Mr. W. T. L. Travers during the visit of H.M.S. 'Challenger' to Wellington, and Moseley was able to describe them in detail externally, after dissection and in transverse section; but he made no longitudinal sections owing to lack of material, with the result that the internal structure could be only partly worked out. Two years later Hutton (8) published a list of the New Zealand planarians which had so far been described, and gave a brief description of the characteristic external features of each species. This list included three marine forms as well as Geoplana traversii mentioned above, and in the following year Hutton (9) added to this list two new species which he himself had discovered—Geoplana moseleyi, a small worm about 27 mm. in length in a preserved specimen, and Rhynchodemus testaceus of the generic identification of which he was then doubtful.

No further work was recorded until 1895 when Dendy (3) published the first of a series of notes on the New Zealand Planarians in which he described the external appearance, shape, and colour of several new species including Geoplana

triangulata, a large worm from Christchurch with a variety australis especially common in Dunedin. Later, in 1897, Dendy (4) referred to this same variety a preserved specimen which had been sent to him from Nelson by Sir James Hector (6) who had recorded it in 1893 but had not been able to identify the worm at that time. Dendy's nomenclature was based entirely on external characters.

Von Graff (5) included Geoplana triangulata in his monograph but did not add anything further to the description given by Dendy, merely quoting extensively from the original text. Up to the present, occasional papers have been published in the 'Trans. N.Z. Inst.', describing new planarians, but all such classification has been based entirely on external characters and no work has yet been done on the internal anatomy of any planarian occurring in New Zealand. In his monograph von Graff (5) erects a new genus, Artioposthia, for such forms of the family Geoplanidae as have muscular gland-organs in connexion with the genital atrium. As I find these present on dissection and on examining serial sections of this worm, I attribute Dendy's species to von Graff's genus Artioposthia.

ECOLOGY.

There seems to be no definite limitation of locality for the two varieties of Artioposthia triangulata as assumed by Dendy. Specimens of the 'type' were collected in Dunedin, not Christchurch, while the variety was found to be equally abundant in both Dunedin and Christchurch, and not confined to Dunedin as stated by Dendy.

While this planarian undoubtedly lives in the soil, it is more readily found under boxes, sacks, tins, or any large object which has been lying undisturbed on the soil for some time. Here the soil must be moist and the worm is usually found in association with slaters, slugs, and centipedes. In moist soil of a clayey nature the worm was found several inches below the surface. In one garden in which this worm was known to occur, none was found after extensive digging in the more dry loamy parts.

On turning over the boxes, &c., I found the planarian on the surface of the soil in a very characteristic coiled position, with

the ventral surface pressed firmly against the soil so as to form a flat spiral outlined by the pale lateral bands of the dorsal surface. In captivity the planarian still assumed that position, lying on the surface of the soil with the anterior and posterior ends buried.

MATERIALS AND METHODS.

For micro-dissection it was difficult to find a method of killing this planarian in an extended position without rendering the worm too soft and friable. Following the recommendation of Ullyot (15) I tried Steinmann's (14) fluid—a mixture of nitric acid, mercuric chloride, and sodium chloride—which certainly killed the worm with practically no muscular contraction, but the tissues were all rendered very brittle and inclined to absorb water, resulting in distortions which made accurate dissection almost impossible. Very weak alcohol gradually increased in strength proved to be more efficient, but in the alcohol, however weak, the worm in its contortions often ruptured the epidermis, causing a certain amount of internal displacement of some of the organs.

For histological work Carleton's (1) method of killing and fixing trematodes was used quite successfully. The worm was placed between two glass plates loosely tied near each end, and left for a short time in that position until it was fully extended. It was then quickly lowered into a dish containing mercuric chloride and acetic acid, heated to 50° C. This resulted in rapid fixation without much apparent shrinkage. After the removal by iodine of any mercury deposit the worms were stored in 70 per cent. alcohol till required. The internal anatomy and histological structure were studied in serial sections which were cut in different planes. As the worm was too large to sectionize completely, horizontal and transverse series of sections were made of the following regions. the anterior end, the pharynx, the genital organs, and the posterior end.

Various staining methods were tried, such as haematoxylin and eosin, picro-indigo-carmine, methylene blue, but except in the case of haematoxylin mounted in euparal 'vert' which clearly demonstrated the nuclear structure, none of the above stains was so successful as borax-carmine counter-stained with

picro-nigrosin which later I adopted as the universal staining method.

The external characters of Artioposthia triangulata correspond with the general description given by Dendy (3) for Geoplana triangulata. This is a particularly large worm, a well-grown specimen measuring as much as 8 inches in length, while an average length is 5-6 inches. The worm is narrow and strap-shaped and when crawling elongates itself greatly, the body becoming correspondingly narrower.

The dorsal surface can be distinguished from the ventral by its strong convexity, whereas the ventral surface is flattened; the colour and markings are also quite different on the two surfaces The dorsal surface of the living worm is a dark purplish-brown colour for the median three-quarters of the width, with marginal bands of pale yellowish dotted with minute specks of the same colour as the median portion. The anterior tip is orange-pink, which colour continues back along the marginal bands for about 1 inch, where there are only a few dark specks. Throughout the length of the dorsal surface there is a narrow mid-dorsal line of a dark purple colour. Dendy (3, p. 178) describes this in the posterior portion only, but in all the specimens here examined the line was definite for the whole length. The ventral surface is pale yellowish in colour, thickly peppered with purplish specks similar to those on the marginal bands. There are two apertures on the ventral surface: (1) the pharyngeal aperture, in the mid-ventral line about three-fifths of the length of the worm from the anterior end, and (2) the common genital aperture about half-way between the pharyngeal aperture and the posterior end. Several specimens were found of a variety of this worm which appeared to be much more common than the type and which differed slightly from the type in external markings, such differences being constant for all specimens of this variety. Similarly, differences were found in the internal structure, but so slight were these that without the external differences they could have been regarded as varying stages in the maturity of the worm. This variety differs from the type in the following external features: the marginal bands

are more definitely marked, and stand out clearly from the
median portion, owing to the dark specks being not nearly so
plentifully distributed over the bands as in the type. The specks
are, moreover, confined to a narrow strip along the inner edge
of the marginal band, leaving an almost uniform yellowish strip
along the outer edge. The ventral surface has a definite purplish
appearance owing to the dark specks being so numerous as
almost to obscure the light background.
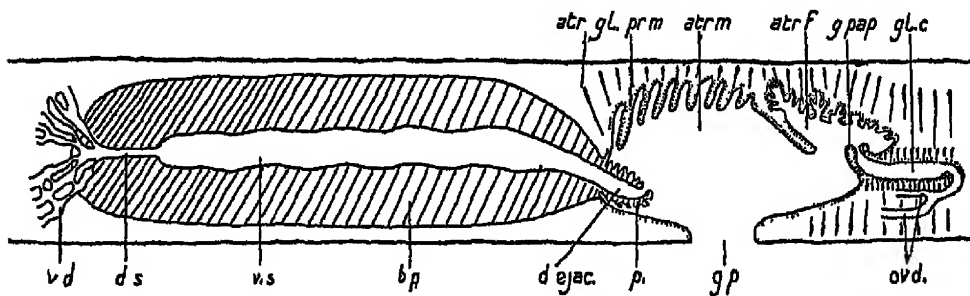
TEXT-FIG. 1.

Longitudinal vertical section through the genital pores. For
lettering see p. 124.

The small differences in internal structure I shall mention
later.

I have seen preserved specimens of this variety in which the
marginal specks and those on the ventral surface had lost their
colour, leaving only the pale background. Dendy (3, p. 180)
describes a variety australis in which 'speckles are absent
on the marginal bands and on the ventral surface', so that it is
quite possible that he is describing this same variety, as I have
not seem any living specimen to which this description applies.
In any case, since the differences between the variety and the
type are so small, it is possible that they are merely due to
seasonal changes in the one type of worm.

REPRODUCTIVE ORGANS.

I. General.

The common genital pore occupies the normal position on
the ventral surface, between the pharynx and the posterior end,
being a little nearer to the pharynx than to the posterior end.
The genital pore leads into the atrium masculinum (Text-fig. 1)

in the anterior portion of which lies the penis. Posteriorly the atrium masculinum receives the atrium femininum into which the female duct opens.

II. Male Organs.

(a) Testes.

The numerous testes (fig. 1, Pl. 14) form two longitudinal lateral zones which extend from behind the brain to the region of the glandular canal (fig. 27, Pl. 16) of the female organs. They occupy a ventral position in the body and lie immediately dorsal and lateral to the nerve-cord, but often so close to it that ventral portions of the testicular sacs may be found among the network of nerves. Each testis (fig. 2, Pl. 14) is more or less spherical in shape and is enclosed in a tunica propria of flattened epithelial cells with bulging nuclei. The cavity of the testis is filled with spermatozoa in all stages of development, derived from a germinal epithelium of sperm mother-cells. Dendy (3) in the testis of Geoplana spenceri describes a space between the outer sperm mother-cells and the central compact mass of developing spermatozoa. This space is no doubt due to shrinkage of the central mass during fixing and preserving, as I found it in only a few of my sections, while in others the developing spermatozoa extended out to the sperm mother-cells.

The spermatozoa when ripe pass into the vasa efferentia, where their structure may best be seen. The head is small, rounded, and highly refringent and with borax-carmine stains a deeper pink than the tail.

(b) Sperm Duct.

The vasa efferentia (figs. 2 and 4, Pl. 14) are extremely slender canals and arise as direct prolongations of the ventral walls of the testes. After a very short course they lead into collecting vessels, the vasa intermedia (figs. 3 and 4, Pl. 14) in which the spermatozoa which were scattered in the vasa efferentia become more densely packed together so that it is fairly easy to follow the course of these vessels on account of their deeply staining contents. The vasa intermedia are slightly convoluted and form a network lying in the upper portion of the circular muscles of

the body-wall, just ventral to the nerve. They are of varying length and ultimately open into the vasa deferentia (figs. 1 and 3, Pl. 14). These last (Text-fig. 2) form a very conspicuous part of the worm, and consist, on each side, of a series of wide, convoluted, branching tubes extending from the region of the pharyngeal aperture to the anterior end of the seminal vesicle (fig. 5, Pl. 14, and fig. 20, Pl. 16). They occupy a ventral position
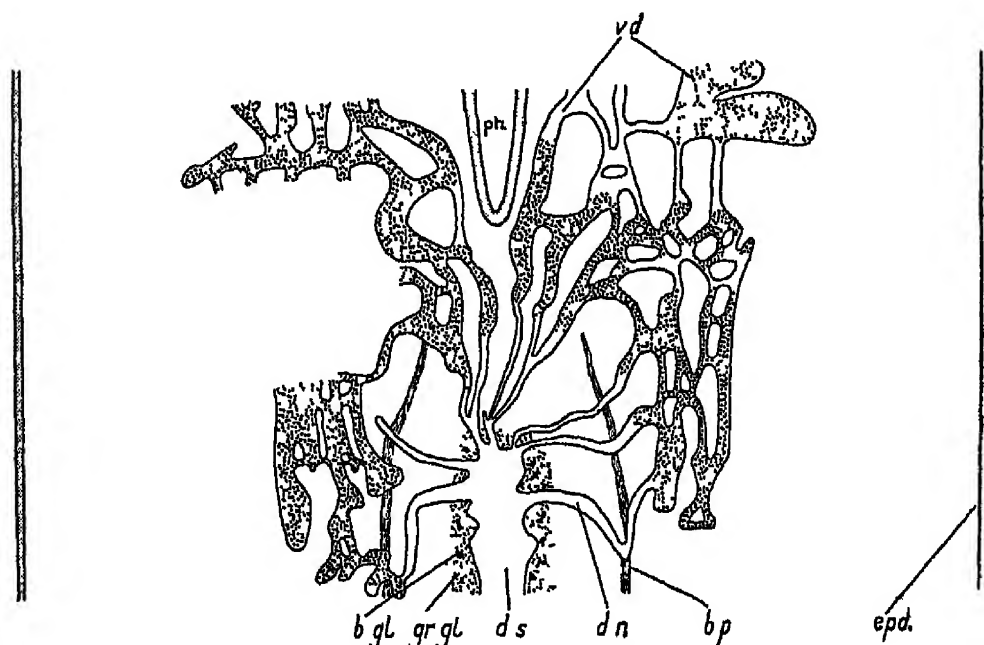
TEXT-FIG. 2.

Longitudinal horizontal section, showing network of vasa differentia opening by narrow ducts into the seminal duct, *d.s.* For lettering see p. 124.

in the body lying dorsally to the nerve-cord, and in the ripened condition the branches extend for almost the whole width of the worm. The vasa deferentia are capable of great distension and form a reservoir for storing the spermatozoa.

As far as I can find in the literature available to me, no such branching vas deferens has been described for any land-planarian. In this group the posterior end of the vas deferens is normally convoluted and swollen, often to a great extent, but nowhere is there any description of branches. This branching system was quite apparent in worms under a dissecting microscope (fig. 5, Pl. 14). Three separate series of sections of this

region were cut, and reconstruction drawings made of the complete system. Of the variety one series was cut horizontally (Text-fig. 2) and one transversely to the long axis of the worm. In these the branches were not greatly distended, so that there was ample evidence of the branching, with wide spaces between the branches. The third series was a transverse one of the type, and here the worm was obviously more mature in that the vasa deferentia were so swollen and distended as to occupy almost the whole width of the worm with very narrow spaces between the branches.

The testicular sacs are much more numerous and closely aggregated in front of the vasa deferentia, but those scattered sacs which lie adjacent to the vasa deferentia open directly into them without either vasa efferentia or vasa intermedia.

The vasa deferentia of each side converge posteriorly and give origin to four to six narrow ducts (*d.n.*, Text-fig. 2; figs. 20 and 21, Pl. 16) which are straight tubes lying almost in one plane. As a rule they do not store spermatozoa. These narrow ducts, which I consider to be still part of the vasa deferentia, enter a median canal surrounded by the musculature of the penis-bulb (*d.s.*, Text-figs. 1 and 2; fig. 21, Pl. 16). This median canal I call the seminal duct as it leads from the vas deferens into the seminal vesicle. There seems to be no definite symmetry or regular arrangement of the narrow ducts as they enter the seminal duct, some joining together before entering, others entering separately. The seminal duct continues back as a narrow tube for a short distance and then widens suddenly to form the seminal vesicle (*v.s.*, Text-fig. 1, fig. 5, Pl. 14) surrounded by the characteristic loose basket-work of muscle (*b.k.m.*, figs. 20 and 22, Pl. 16) which extends from the thick muscular walls of the seminal vesicle to the outer wall of the penis-bulb. The width of the seminal vesicle and the amount of folding of its walls depend on the degree of maturity of the specimen; but in a fully mature worm the seminal vesicle may extend in width almost to the wall of the penis-bulb.

Von Graff (**5**, p. 163) describes the posterior part of the vas deferens which becomes swollen with stored spermatozoa as forming an outer seminal vesicle. This he calls 'false' to

distinguish it from the 'true' seminal vesicle which has its own strong musculature and therefore preserves a certain constant form. This is readily understood in those species with only one pair of long convoluted vasa deferentia in the posterior ends of which the spermatozoa are stored. But in Artioposthia triangulata, in which the whole of the vas deferens on each side forms a branching network, such a term as 'false' seminal vesicle is not applicable. I reserve, therefore, the term seminal vesicle for the single median tube surrounded by a definite musculature of its own which leads on from the seminal duct.

The seminal vesicle has an approximate length of 9·6 mm. and passes gradually into the ductus ejaculatorius (Text-fig. 1, and fig. 20, Pl. 16) which traverses the penis. The dorsal wall of the ductus now becomes thick and deeply indented, while the ventral wall loses its musculature and flattens out as the duct enters the penis which projects into the anterior end of the atrium musculinum.

Histology of the above Organs.

The wall of the vas efferens (fig. 2, Pl. 14) has the same simple structure as the testicular sac of which it is a direct prolongation. It is formed of flattened epithelial cells with elongated bulging nuclei. In a stained section there was no evidence of cilia, though von Graff (5, p. 162) describes them as being present. Dendy (2, p. 88) evidently does not find them in Geoplana spenceri, and of the more recent writers the few who describe the structure of the vasa efferentia do not find cilia. If they are present in this worm they would be recognizable, as I found cilia elsewhere in preserved material.

The vasa intermedia have slightly thicker walls than the vasa efferentia, with larger nuclei and no cilia. No muscular layer was found in either the vasa efferentia or the vasa intermedia.

In the vas deferens (fig. 6, Pl. 14) the wall is still thicker, but the thickness varies with the distension of the tubes by the contained spermatozoa. Surrounding the epithelium is a thin layer of circular but no longitudinal muscles. Von Graff (5, p. 163) describes the epithelium of the vas deferens as being scarcely half as high as that of the oviduct with round nuclei

not so thickly pressed together as in the oviduct. To quote the exact words: 'In der That ist das Epithel derselben kaum halb so hoch als jenes der Oviducte, die Kerne sind rund und nicht so dicht gedrangt wie in diesen.' This description does not apply here as the epithelium in both the distended and non-distended portions is more than half the height of that of the oviduct (fig. 10, Pl. 15), while the nuclei are elongated not rounded. Very long cilia were easily seen in the parts with no spermatozoa, but were sometimes obscured in those parts which contained the massed spermatozoa, still we may assume that they are a constant feature of the whole of the vasa deferentia.

According to von Graff (5, p. 163) the vas deferens in the front part of the body has no 'muscularis', but muscles appear in it in the region of the pharynx and increase towards the penis. This corresponds with the condition found in Artioposthia triangulata in which the anterior ducts (vasa efferentia and intermedia) have no muscular wall, whereas the vasa deferentia which begin in the region of the pharynx have a definite circular muscle-layer which becomes thicker in narrow ducts at the posterior end.

The narrow ducts are lined with cubical cells with round nuclei and very long cilia, and as they enter the seminal duct the epithelial cells become much longer and narrower until in the seminal duct itself (fig. 9, Pl. 15) they become columnar and are almost thread-like with long compressed nuclei. This characteristic shape may, perhaps, be related to the pressure of the secretion of the male accessory glands which I shall describe later.

The epithelium of the seminal vesicle (fig. 7, Pl. 14) is secretory in function, and the cells are large and very striking in appearance. They are bluntly conical with the distal portions of the cells not in contact with one another. The nucleus and cytoplasm are confined to the proximal part of each cell while the distal region stores the secretion which may be poured into the cavity of the seminal vesicle through the notched apex of the cell. This secretion consists of mucin, as on testing with muci-carmine it took the characteristic violet shade. The wall of the seminal vesicle is very muscular (fig. 7, Pl. 14, and fig. 22, Pl. 16)

with a layer of circular muscles just outside the epithelium
followed by alternate layers of longitudinal and circular muscles.
The whole forms a very firm muscular wall to the seminal
vesicle which in its turn is surrounded by the basket-work of
muscle connected with the outer wall of the penis-bulb.

In the ductus ejaculatorius (fig. 8, Pl. 14) the muscular wall
is not so clearly marked off, and the secretory cells of the seminal
vesicle are replaced by ciliated cells which at the free end of
the penis merge into the ciliated epithelium of its outer surface.

(c) Penis.

The penis (fig. 5, Pl. 14) is a very small, inconspicuous, muscular
organ the wall of which is transversely folded and is no doubt
capable of great extension. It is traversed by the ductus
ejaculatorius, the external opening of which is in the form of
a wide transverse slit lying nearer the ventral than the dorsal
surface, and discharges the male products into the atrium
masculinum.

(d) Atrium Masculinum and Adenodactyli.

The atrium masculinum presents some peculiar features
which I have not found described in such literature as is avail-
able to me. Viewed from the ventral surface (fig. 5, Pl. 14) the
atrium is triangular in shape with the base of the triangle toward
the seminal vesicle. The dorso-lateral walls on each side bear
three thick, rounded, muscular masses or adenodactyli (von
Graff) which project into the atrial cavity leaving between them
only a long narrow vertical space. Ventrally and ventro-
laterally the adenodactyli are not attached to the wall of the
atrium (Text-fig. 3; fig. 24, Pl. 16) which in this region is con-
sequently quite wide—about half the width of the worm.
Anteriorly the adenodactyli of the two sides are joined by a firm
muscular archway (fig. 5, Pl. 14) which curves over the penis,
while posteriorly they converge and join to form a kind of
shallow muscular scoop (sc.m.) which projects freely backwards
and marks the posterior limit of the atrium masculinum.

Each of the adenodactyli is made up of strong wide bands of
muscle which are loosely woven together and enclose a glandular

reservoir (Text-fig. 3; fig. 24, Pl. 16). This is a wide, elongated cavity slightly constricted in the middle and leading at one end into a winding duct which opens into the atrial cavity. The reservoir is lined with curious elongated secretory cells (fig. 11, Pl. 15) arranged in groups of varying heights. These cells have round nuclei near the free end below the terminal secretion which is discharged into the cavity of the reservoir. The wall of
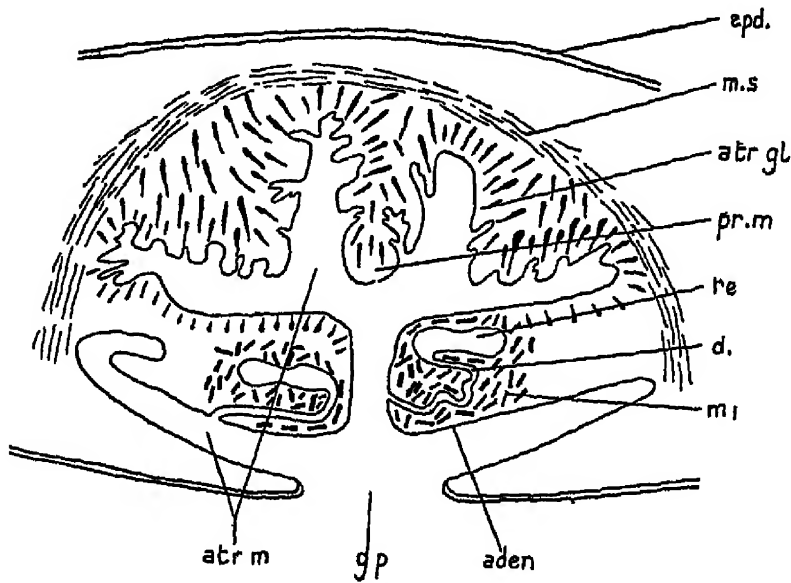
TEXT-FIG. 3.

Transverse section through the genital pore. For lettering see p. 124.

the duct is formed of cubical cells with short cilia and rounded nuclei. These cubical cells take a bright orange stain with picro-nigrosin and appear also to contain secretory granules.

This description of the adenodactyli and their glands agrees in all essential points with that of von Graff (p. 179) for the adenodactyli of Artioposthia, with this important exception that von Graff does not describe a duct leading from the reservoir to the atrial cavity, nor does he figure such a duct in his plates. He describes a flask-shaped or pear-shaped gland which is both a reservoir and a duct and yet the secretion seems to remain in the reservoir, or perhaps is distributed to the surrounding muscular mass. I will quote von Graff's (5, p. 179) description of the gland and its cell structure:

"Es handelt sich namlich bei allen diesen Organen um

flaschen- oder birnformige Drusen, die von einem kraftigen Muskelmantel umgeben sind. Bei den Artioposthia-Arten besteht die Druse stets aus zweierlei Zellen: Kleinen cubischen (meist) Cilien tragenden Epithelzellen, welche das centrale, zugleich als Reservoir und Ausfuhrungsgang dienende Lumen der Druse bekleiden, und birnformigen secretorischen Zellen. Letztere finden sich rings um das Drusenlumen und besonders massenhaft um den Fundus desselben angehauft und convergiren mit ihren Ausfuhrungsgangen, um zwischen dessen Epithelzellen einzudringen und das kornige Secret in das centrale Reservoir zu entleeren."

I find this description very vague and difficult to picture. If the secretion is discharged by the cells into the reservoir it would seem to require a duct to carry the secretion from the reservoir to the exterior, and yet I can find no such duct described or figured. In Artioposthia triangulata the reservoir is lined with secretory and the duct with ciliated cells. Lucy M. Wood (16, p. 612) in describing the male adenodactyli of Artioposthia harrisoni says, 'In transverse section they show a central cavity surrounded by gland-cells which are enclosed in a muscular sheath.' Again there is no duct, and one may wonder what ultimately becomes of the glandular secretion which is poured into this central cavity by the surrounding gland-cells. It can serve no definite purpose unless it reaches the outside of the adenodactyli, and that can only be effected by means of a duct from the central cavity to the atrium.

Apart from this difference the adenodactyli of Artioposthia triangulata with their enclosed glands agree essentially with von Graff's description. Although in the normal condition of this worm the adenodactyli appear as rounded masses, and do not project through the genital aperture as seems to be the case with the majority of male adenodactyli, yet they are quite extensible and have been observed to be thrust out into the atrial cavity as elongated processes enclosing the reservoir and duct. In this respect they resemble more the female adenodactyli of Artioposthia fletcheri (5, p. 227) which do not project so far into the atrial cavity.

As for the function of the adenodactyli, one cannot make

a definite statement until one has made a careful study of the habits of this worm. One can only conjecture from the small size of the penis that the adenodactyli with their great muscularity do in some way supplement the function of the penis.

The dorsal wall of the atrium masculinum, i.e. the tract between the dorsal portions of the adenodactyli, bears along its length an irregular series of long, narrow, muscular processes (*pr.m.*, Text-fig. 3; fig 5, Pl. 14; fig. 24, Pl. 16) which are smaller than the adenodactyli and have not their complex glandular structure.

The atrium masculinum is lined with a single-layered epithelium of cubical ciliated cells continuous with the outer epithelium of the penis. There appears to be no definite muscle-layer lying immediately underneath the epithelium as is generally the case, but closely interlacing muscle-fibres are found in the parenchyma outside the epithelium, and these no doubt serve the same purpose.

(e) Accessory Glands.

Two glands are found in association with the seminal duct and seminal vesicle. These lie in the parenchyma surrounding the duct, and may be differentiated according to the form of the secretion and the colour which they assume on staining.

(1) A 'diffuse' gland (*b.gl.*, fig. 21, Pl. 16) extending from the epithelium of the duct right out to the wall of the penis-bulb. It occurs especially round the seminal duct and to a much less extent round the seminal vesicle. With borax-carmine the secretion takes the same bright red colour as that of the skin glands.

(2) A finely 'granular' gland (*gr gl.*, fig. 21, Pl. 16) which is stained a bright pink with borax-carmine. This gland surrounds only the seminal duct and extends throughout its length. As it passes back along the duct the gland becomes more concentrated until towards the posterior end it forms a deep pink band round the seminal duct (fig 23, Pl. 16), by which time the 'diffuse' gland has become greatly reduced, being found only at irregular intervals behind this region.

The ducts of these glands (fig. 9, Pl. 15) as of the skin glands

I

are merely extensions of the body of the gland-cells which force
their way between the epithelial cells of the cavities which they
surround, and there swell up continuously with the pressure of
the secretion from behind. The epithelial cells are consequently
pressed together so that ultimately they assume a thread-like
form such as we find in the seminal duct. In the seminal vesicle
there is no such modification of the epithelium, as the accessory
glands are very scattered and the main secretion takes place
from the epithelial cells themselves. Stained masses of the
secretion can be seen in the cavities of the seminal duct and of
the seminal vesicle where the secretion performs the usual
function of mixing with the spermatozoa on their way to the
genital atrium.

A diffuse atrial gland similar to that surrounding the seminal
duct and seminal vesicle occurs throughout the parenchyma
of both the dorsal muscular processes and the muscular masses.
The gland-cells take a deep crimson stain with borax-carmine
and prolongations of these cells force their way between the
outer epithelial cells and so empty their products into the atrium
masculinum.

III. Female Organs.

(a) Ovaries.

The ovaries of Artioposthia triangulata are quite
unusual and, in the literature available to me, I can find no
description which is applicable to the condition here found.

There is a single pair of ovaries (fig. 5, Pl. 14; fig. 20, Pl. 16)
which are situated laterally or ventro-laterally in the region of
the seminal vesicle, and lie just outside the posterior limb of
the gut. Each ovary (fig. 5, Pl. 14) is a long fusiform body more
or less regularly constricted on its ventral surface. This fusiform
shape seems to be peculiar to this worm, as von Graff (5, p. 151)
describes the ovary as being 'usually spherical with occasional
oval forms'; Dendy (3, p. 82) finds the ovary of Geoplana
spenceri 'pear-shaped', while Kaburaki (11, p. 145) finds it
'nearly oval' in Geoplana whartoni. It will be shown
later, however, that this so-called ovary of Artioposthia
triangulata is really a complex structure enclosing more than

one true ovary or germarium, as well as specialized parovarian and amoeboid cells.

The position of the ovary in the body is also unusual. As a general rule, in land planarians the ovary is situated far forward, in the region of the brain, and von Graff (5, p. 151) quotes a variety of examples to illustrate this, adding that the posterior boundary of the ovary does not extend beyond the anterior third of the body. In Artioposthia triangulata the ovary is much farther back, its anterior end being 77 mm. from the anterior end of a worm of 152 mm. length, i.e. half-way back. This unusual position of the ovary does not seem to have any bearing upon its relationship with the other genital organs.

The ovary (fig. 12, Pl. 15; fig. 25, Pl. 16) is enclosed in an epithelium of pavement cells with long flattened nuclei, surrounding which is a layer of loosely arranged circular muscle-fibres (c.m.). The main portion of the ovary is made up of parenchyma which, at intervals along the ventral surface, extends from the ovary towards the adjacent wall of the seminal vesicle (fig. 20, Pl. 16). The ovarian epithelium is interrupted at these points of extension, and the circular muscle-fibres follow the course of the parenchymatous band towards the seminal vesicle. In a transverse section (fig. 12, Pl. 15, and fig. 25, Pl. 16) the ovary is seen to consist of an outer area of parenchyma enclosing a more or less definite inner region in which are found the germaria (gm.) and groups of specialized cells connected with the nutrition of the ova (am. and par.).

The germaria (fig. 5, Pl. 14) are situated on the dorsal or dorso-lateral margin of the inner area of the ovary. In two specimens of the variety I found two germaria in each ovary, and in one specimen of the type I found four (as illustrated). ' Without examining a great many worms in all stages of maturity I am not able to state definitely whether the number of germaria is constant and definite for each variety, or whether it varies according to the maturity of the worm. I am inclined to think that the latter is the true explanation, as in the worm in which four germaria were present all the genital organs were in a more advanced stage of development.

When four germaria are present the anterior one is situated

at about one-quarter of the length of the ovary from the anterior end, with the other three arranged at regular intervals behind. The anterior germarium is large and mature and lies directly opposite the internal opening of the oviduct (fig. 12, Pl. 15) which enters the ovary on the ventral surface. The remaining three germaria become successively smaller and more immature until the posterior one is very small and contains only a very few ova. The same relationship is seen in the variety with only two germaria present. The anterior one is large and mature and has the same relative position as in the type, while the second germarium which is quite small lies about half-way along the ovary.

As there is only one oviduct on each side of the worm, with only one opening leading into the ovary opposite the anterior germarium, we may presume either that the germaria as they develop and ripen move forward in turn until they lie opposite the opening of the oviduct, or that after the escape of the ripe ova from the anterior germarium the oviduct withers anteriorly and forms a new connexion with the second germarium, and so on with the others as they in turn ripen.

The germaria (fig. 14, Pl. 15, and figs. 25 and 26, Pl. 16) are pear-shaped sacs which lie with their broad ends along the outer margin of the inner area of the ovary and their pointed ends towards the centre. There is a definite tunica propria of flattened epithelial cells (fig. 14, Pl. 15), surrounding which are a few irregularly placed muscle-fibres. This description of the germarium may be compared with that of Moseley (12, p. 186), for the ovary of Bipalium, which is pear-shaped with a 'distinct but delicate membranous capsule' and 'externally to the capsule a wide space occupied by an irregular mesh-work of connective tissue'—this latter corresponding with the outer area of parenchyma in the ovary of Artioposthia triangulata. In Artioposthia the germarium is lined with a germinal epithelium (fig. 14, Pl. 15) continuous with an inner irregular mesh-work of branched cells forming a stroma in which are embedded the ova. The nuclei of the stroma cells are exactly similar to those of the germinal epithelium, being oval in shape with deeply staining chromatin, which bears out von

Graff's statement (5, p. 151) that the stroma cells are differentiated germinal epithelial cells and not parenchyma.

In the young oocytes next to the germinal epithelium one can see quite clearly the various phases of meiosis through which the nucleus is passing—definite chromosomes, bouquet stage, &c. As the ova grow and increase in size, the nucleus becomes larger and vesicular with one deeply staining nucleolus in the chromatin network. Yolk-bodies are formed in the cytoplasm, at first only one or two which increase to as many as ten in a mature ovum. These yolk-bodies are not composed of fat or oil but take a protein stain.

The immature germarium is similar to the mature in general structure, but has only a few large ova occupying the central part of the mass. In a mature germarium spaces may sometimes be observed around the ova or to one side of them. These spaces are probably what Moseley (12, p. 137) describes as 'egg-capsules'; but are no doubt here, as in Moseley's specimens, due to the shrinkage of the cytoplasm of the ova, as they are present in some of my preparations and not in others

Von Graff states that at the end of the functioning period the epithelial cells and stroma cells disappear, the latter being used to form yolk-bodies in the ripe eggs. This condition does not exist in Artioposthia triangulata, as in the mature germarium (fig. 14, Pl. 15) the germinal epithelium and stroma cells are quite as large and numerous as in a less advanced germarium, unless such a reduction takes place at the very end of the functioning period just before the ova leave the germarium.

Dendy (2, p. 82) describes in Geoplana spenceri spindle-shaped cells which appear after the formation of the young ovum and adhere closely to its surface. These spindle cells later on disappear when the ovum becomes mature. I think that there is no doubt that these spindle cells correspond to the stroma cells of the germarium of Artioposthia triangulata which are present in all the early stages of development, and which, according to von Graff, are gradually absorbed and worked up into the yolk bodies of the ripe ova.

The inner portion of the ovary (fig. 12, Pl. 15, and fig. 25,

Pl. 16) is roughly outlined by a layer of deeply staining cells which are connected with rows and groups of similar cells in the inner parenchyma. These deeply staining cells I shall call parovarian cells for reasons which I shall give later. The inner parenchyma is loosely arranged to form a number of irregular cavities in which are found isolated cells or small clumps of two or three cells which I shall call amoeboid cells on account of their characteristic shape. These two types of cells constitute the specialized cells of the inner area of the ovary and I shall now describe each type in detail.

The parovarian cells (figs. 17 and 18, Pl. 15) present a variety of shapes from cubical cells with large round nuclei to elongated ones with long narrow nuclei. They are immediately noticeable in any preparation stained with Borax-carmine on account of the brilliant red colour taken by the cell inclusions. What these inclusions are I cannot say, but sometimes they have the form of fine granules, at others they appear as a tangled mass of very finely looped threads. The nucleus is in some cases small and round at the base of the cell, in others, large and vesicular and in the centre. These parovarian cells seem to be constantly dividing amitotically as Woodworth (17, p. 33) describes for Phagocata gracilis, and one can see the elongated dumb-bell-shape of the nucleus so characteristic of amitotic division. The parovarian cells ultimately become continuous with similar cells arranged as a wall to a tube (fig. 12, Pl. 15, and figs. 25 and 26, Pl. 16) which leaves the germarium at its inner pointed end and leads towards the internal opening of the oviduct. This parovarian tube arises on the inner side of the germarium as a small papilla outlined by stroma cells which may be compared with the papilla described by Moseley (12, p. 138) for Rhynchodemus as follows: 'In Rhynchodemus the oviduct was found to take origin from a papilla on the upper and inner side of the ovary and projecting into its cavity. The papilla is formed of spindle-cells and a number of similar cells are to be found scattered in the loose tissue around its base.'

As the parovarian cells which lead from the germarium have especially deeply staining inclusions, it may be assumed that these inclusions are for the nutrition of the ova and will be

discharged into the oviduct. I have repeatedly observed in the first portion of the oviduct small deeply staining granules which may be presumed to have come from the parovarian cells of this region.

Von Graff (5, p. 152) describes two types of parovarium: (1) an appendage which opens into the oviduct immediately after it leaves the ovary, and which is really a mass of yolk. This may also extend outwards and give rise to the main yolk-glands; and (2) an appendage which is exactly similar to the ovary in structure and may be considered to be merely an abnormal diverticulum of the ovary. The parovarian cells of Artioposthia triangulata belong to type (1) though they are not massed together to form a solid gland, but are arranged in rows of cells.

According to the same authority the parovarium has two connexions with the oviduct; one, directly, as the oviduct leaves the ovary, and the other, indirectly, through the main yolk-glands which arise from the parovarium and which open into the oviduct at intervals along its length. I can find no connexion between the parovarian cells of Artioposthia triangulata and the main yolk-glands. In all the specimens which I examined, the yolk-glands were well developed, and if they had arisen from the parovarian cells, as von Graff suggests frequently happens, there would I think have been some evidence of this connexion here.

Woodworth (17, p. 33) also describes for Phagocata gracilis the chains of yolk-cells leading from the parovaria to form rudimentary yolk-glands which branch to form 'a dendritic system of rapidly dividing cells which ramify through the tissues'. He considers that this is the normal origin of the yolk-glands which is so clearly shown in Phagocata, but which may not be so evident in another genus where the cells of the yolk-gland and of the parovarium are more differentiated and therefore are not so obviously related. But even then one should be able to find some evidence of a connexion between the two groups of cells, and I entirely failed to do so after careful examination of stained serial sections.

Von Graff, although he does not appear to have looked into

this matter himself, in reviewing the evidence of research workers to date, is not so definite as Woodworth in his statements, and in all the specific examples he quotes there is no mention of a direct connexion between the parovarium and the yolk-gland. I cannot but believe that, in Artioposthia triangulata at least, the parovarium and the yolk-gland develop separately and each forms for itself its own connexion with the oviduct.

The amoeboid cells are to be found in irregular cavities in the inner parenchyma (fig. 12, Pl. 15) either isolated, or in clumps, or lining the walls of these cavities, so that it would appear as if the cells developed from the parenchymatous tissue and dropped into the cavities when mature. A single cell (figs. 15 and 16, Pl. 15) has the characteristic irregular shape with a large nucleus, and in the cytoplasm a clear non-staining vesicle about the same size as the nucleus. The vesicle probably contains a reserve product of an oily nature which has been dissolved out in the process of fixing and staining, and which may help in the nutrition of the egg as these cells pass into the oviduct with the ova.

The oviduct on entering the ovary on its ventral surface passes through the outer parenchyma and widens to form the internal opening (fig. 12, Pl. 15) just inside the inner parenchyma The germarium lies almost opposite this on the dorsal wall and the connexion between the germarium and the internal opening of the oviduct is as follows. The parovarian tube which leads away from the germarium ends abruptly in a cavity of amoeboid cells which does not seem to be definitely outlined and which is connected with other similar cavities in the central portion of the ovary. From here a short tube containing amoeboid cells enters the internal opening of the oviduct from which it can be distinguished by the fact that the oviduct is ciliated, and almost encloses the tube of amoeboid cells (fig. 13, Pl. 15). The fact that the oviduct does not leave the germarium itself gives both the parovarian and the amoeboid cells an opportunity either of discharging their nutritive products into the oviduct (as in the case of the former) or of themselves entering the oviduct (as in the case of the latter).

(b) Oviducts.

The oviduct on each side arises ventrally from the ovary about one-quarter of its length from the anterior end, and continues as a narrow tube straight back towards the posterior end of the worm lying just dorsally to the longitudinal nerve (fig. 5, Pl. 14). After passing the atrium femininum the oviducts converge slightly towards each other, and then turn abruptly at right angles to meet in the middle line (Text-fig. 1). This common duct continues back for a very short distance, and then turns forward to enter the posterior end of the glandular canal (fig. 5, Pl. 14). The oviduct is round in section and is lined with short columnar cells with long cilia (fig 10, Pl. 15). Surrounding the epithelium is a layer of circular and longitudinal muscles.

(c) Glandular Canal.

The glandular canal runs forward from the single oviduct to the posterior end of the atrium femininum which it enters on a small papilla on the left (Text-fig 1, and fig. 5, Pl. 14) The canal (fig.'27, Pl. 16) is twice the width of the oviduct in section, but the epithelium is folded so as to be capable of much greater extension. The epithelium is formed of ciliated columnar cells, surrounding which is a layer of circular and longitudinal muscles.

In a section stained with borax-carmine (fig. 27, Pl. 16) the whole of the wall of the canal as well as the parenchyma for some distance round it appear as a bright red mass. This is due to a glandular secretion which is present in such large quantities as to obscure the tissues through which it passes. The glands themselves are scattered throughout the width of the worm and open into the glandular canal along its whole length, the secretion no doubt being used in the formation of a cocoon.

(d) Yolk-glands.

The yolk-glands (figs. 20 and 21, Pl. 16) are found throughout the length of the worm occupying the spaces between the diverticula of the alimentary tract. The gland on each side consists of irregularly branched groups of cells (fig. 5, Pl. 14),

each group constituting a follicle (fig. 19, Pl. 15) which, however, has no enclosing membrane. The cells are large and roughly hexagonal from mutual pressure, and each cell has a thick firm cell-wall which encloses a mass of highly refringent spherical deutoplasmic bodies—the yolk spheres. The nucleus is large, oval, and granular and is situated at one side of the cell.

As described by von Graff (5, p. 155) the glands at first appear as single cells scattered throughout the parenchyma, these cells by division forming smaller and then larger groups of cells until the complete gland is formed. The young cells (fig. 19, Pl. 15) are situated on the periphery of the follicle and are smaller than the mature cells and of a variety of shapes. With haematoxylin and eosin the granular cytoplasm takes a uniform deep violet colour and encloses a large granular nucleus with a deeply staining karyosome.

Since the yolk-glands extend from one end of the worm to the other, and since the oviduct in Artioposthia triangulata is shorter than in other worms of this group where the ovary is far forward, it remains a still greater problem how the yolk-granules from the anterior and posterior regions of the worm find their way into the oviduct. At regular intervals along the oviduct (fig. 5, Pl. 14) appear short stumpy branches, each of which connects with an adjacent yolk follicle. The enclosed cells break down when ripe and their contents are directed into the oviduct by the ciliated epithelium of the branch. Neighbouring masses are able to dispose of their products through the cavity left by the yolk follicle which has already discharged its products into the oviduct. Since the yolk-masses are connected throughout the worm, and since these are more mature in the region of the oviduct than at the ends of the worm, it may reasonably be assumed that the unripe yolk-masses move up to take the place of the ripened ones which have already discharged their contents into the short branches of the oviduct lying alongside. Such a movement of yolk could be caused by the constant contraction and expansion of the gut diverticula during the passage of food through the worm and by the muscles of the body generally.

No receptaculum seminis and no uterus were found.

(e) Atrium Femininum.

The atrium femininum (Text-fig. 1 and fig. 5, Pl. 14) is much smaller than the atrium masculinum, being less deep dorso-ventrally and only about one-quarter of its length. It tapers posteriorly where it receives on the left side a small papilla on which opens the glandular canal. The atrial cavity is lined by ciliated epithelium and the dorsal wall bears irregular muscular processes which, however, have none of the characteristic features of adenodactyli.

SUMMARY.

The main subject of this paper is a detailed description of the reproductive organs of a planarian initially described by Dendy as Geoplana triangulata. Five unusual features are observed in the reproductive system.

1. The vas deferens consists of a series of wide convoluted branching tubes extending from the region of the mouth to the anterior end of the seminal vesicle.

2. The penis is very small and inconspicuous.

3. The atrium masculinum is provided with three pairs of muscular gland-organs or adenodactyli.

4. The paired ovaries are situated one on each side of the seminal vesicle, not in the region of the brain as is usual.

5. Each ovary is a long fusiform body enclosing more than one true ovary or germarium, as well as specialized parovarian and amoeboid cells which are probably nutritive, and are associated with the internal opening of the oviduct.

The writer refers Geoplana triangulata Dendy to the genus Artioposthia owing to the presence of adenodactyli in the atrium masculinum. Each adenodactylus encloses a glandular reservoir from which a ciliated duct leads to the atrial cavity. The actual function of the adenodactyli is obscure, but the very small size of the penis and the fact that the adenodactyli are extrusible suggests the possibility of these latter performing the function of a penis.

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Explanation of Lettering.

aden, adenodactylus, *al c*, alimentary canal, *am.*, amoeboid cell, *am.t*, tube of amoeboid cells; *atr f*, atrium femininum; *atr gl*, atrial gland; *atr m*, atrium masculinum, *b gl*, diffuse brightly staining gland of sperm duct; *bk m*, basket-work of muscle; *b p.*, penis-bulb, *c*, cilia, *c ep*, ciliated epithelium; *chs*, chromosomes, *c ı*, cell inclusions of parovarian cells, *c m*, circular muscles, *d.*, duct leading from glandular reservoir to atrial cavity; *d ejac*, ductus ejaculatorius, *d b gl*, duct of brightly staining gland; *d gr gl*, duct of granular gland; *d.n.*, narrow duct, *d.s.*, seminal duct; *ep*, epithelium; *epd*, epidermis, *epd gl.*, epidermal gland, *fol.*, yolk follicle, *ger.ep.*, germinal epithelium, *gl c.*,

glandular canal; *gm.*, germarium; *g p* , genital pore , *g pap.*, genital papilla; *gr gl* , granular gland of seminal duct, *l m.*, longitudinal muscles; *m i.*, interwoven muscle of adenodactylus, *m s* , muscular sheath surrounding genital atrium, *n c* , nerve-cord; *nu.*, nucleus, *nuc.*, nucleolus, *o c m.*, outer circular muscle; *om* , ovum, *ooc* , oocyte; *ov* , ovary; *ovd.*, oviduct, *p* , penis, *pa i* , inner parenchyma of ovary, *pa o* , outer parenchyma of ovary; *pap* , papilla, *par* , parovarian cells, *par t* , tube of parovarian cells , *ph* , pharynx; *ph ap.*, pharyngeal aperture; *pr m.*, muscular processes of atrium masculinum, *re* , glandular reservoir, *sc m* , muscular scoop, *se* , secretion, *se.c.*, secretory cells, *sp.*, spermatozoa; *sp c* , spindle cells, *str* , stroma; *t* , testis, *t pr* , tunica propria; *tr m* , transverse muscle; *vac.*, vacuole, *v d* , vas deferens, *v ef.*, vas efferens, *v i* , vasa intermedia, *v s.*, seminal vesicle, *y.b.*, yolk-body; *y.br.*, branch of oviduct to yolk follicle, *y.c* , yolk-cell, *y' c'* , young yolk-cell, *yk gl.*, yolk-gland; *y.s* , yolk-sphere

DESCRIPTION OF PLATES 14–16.

PLATE 14.

Fig. 1'—Horizontal section of one side of Artioposthia triangulata showing ventral portion of the testes, represented as clear spaces among the branches of the nerve. ×18

Fig. 2 —Section of a single testis with vas efferens ×200.

Fig. 3 —Horiz sect of one side slightly more ventral than fig. 4, to show the course of the vasa intermedia entering the vasa deferentia. ×18

Fig. 4 —Semi-diagrammatic transverse section of ventral portion showing connexion between vasa efferentia and vasa intermedia ×40.

Fig 5 —Drawing of dissection from ventral surface to show main genital organs (semi-diagrammatic). ×5.

Fig. 6.—Transverse section of wall of typical vas deferens ×550

Fig 7 —Transv sect of wall of seminal vesicle Secretion stained with picro-nigrosin ×550

Fig 8.—Transv. sect. of wall of ductus ejaculatorius ×550.

Fig. 9 —Transv. sect. of wall of seminal duct stained with borax-carmine to show two kinds of glands. ×550.

PLATE 15.

Fig. 10 —Transv. sect. of wall of oviduct ×550.

Fig. 11 —Transv. sect of lining of reservoir. Glandular secretion stained with picro-nigrosin. ×550.

Fig. 12 —Transv. sect. of ovary showing the entrance of the oviduct— slightly diagrammatic ×85.

Fig. 13 —Transv. sect. of inner end of oviduct showing entrance of tube of amoeboid cells.

Fig. 14.—Transv. sect. of mature germarium. ×400.

Figs. 15 and 16.—Types of amoeboid cells.

Figs 17 and 18.—Types of parovarian cells.

Fig. 19.—A single follicle of yolk-cells × 40.

PLATE 16.

Fig. 20.—Photomicrograph of a horizontal section of the region between the pharyngeal aperture and the genital pore. × 14

Fig 21.—Photomicrograph of a transverse section of the seminal duct showing two narrow ducts proceeding towards it from the vasa deferentia. × 24.

Fig. 22 —Photomicrograph of a transverse section of the seminal vesicle. × 24.

Fig 23.—Photomicrograph of a transverse section of the seminal duct near its posterior end showing the concentration of the pink granular gland round the duct. × 36.

Fig. 24.—Photomicrograph of a transverse section through the genital pore and two adenodactyli with their enclosed reservoir and ducts × 24.

Fig. 25.—Photomicrograph of a transverse section through the ovary showing inner and outer area of parenchyma, and oviduct leaving the ovary. × 80.

Fig 26.—Photomicrograph of a horizontal section showing portion of the ovary with one germarium and the parovarian tube leaving it. × 170.

Fig. 27.—Photomicrograph of a transverse section through glandular canal showing the extent of the gland which pours its secretion into the canal. × 24.

All the photomicrographs are stained with borax-carmine and picro-nigrosin.
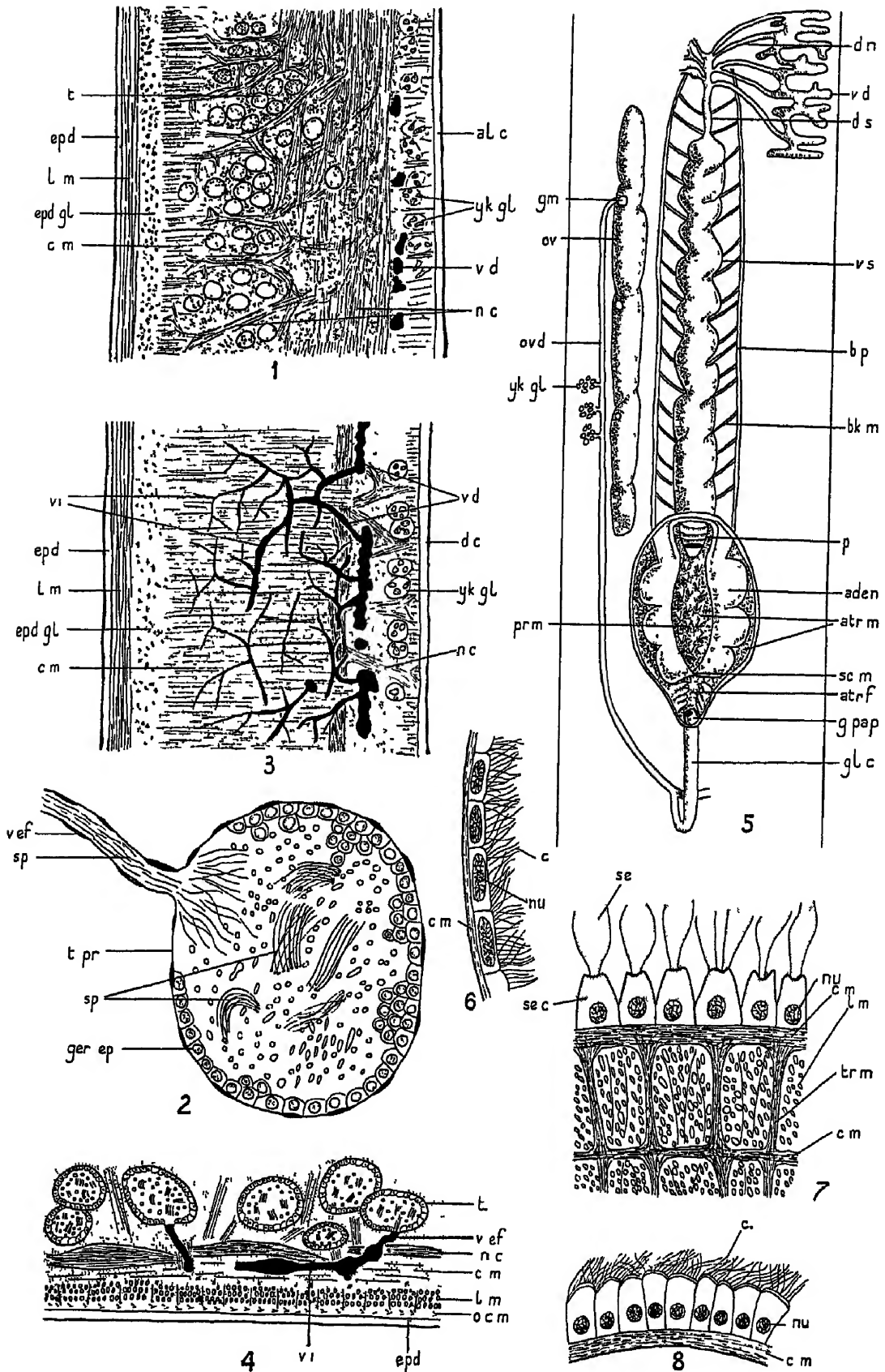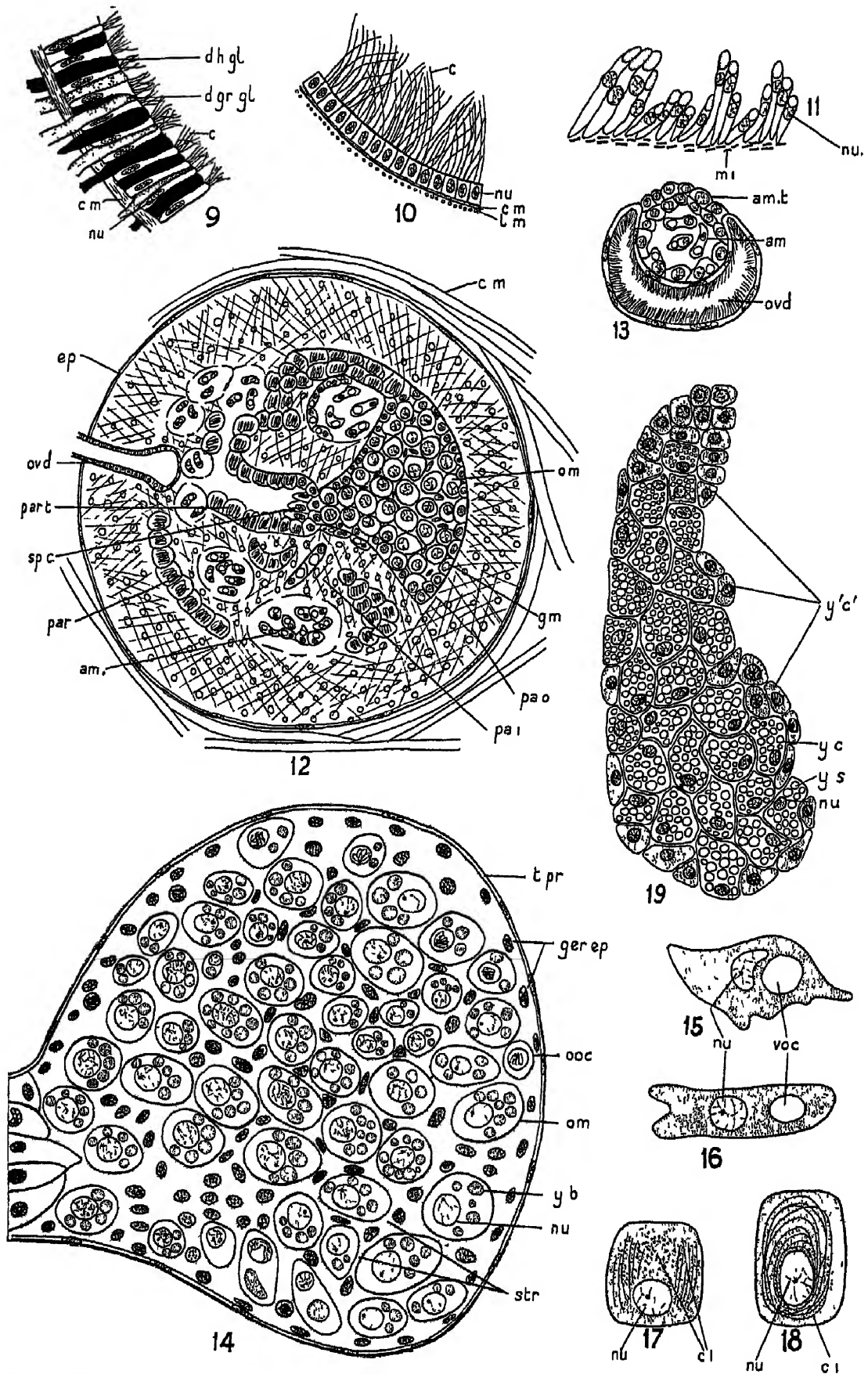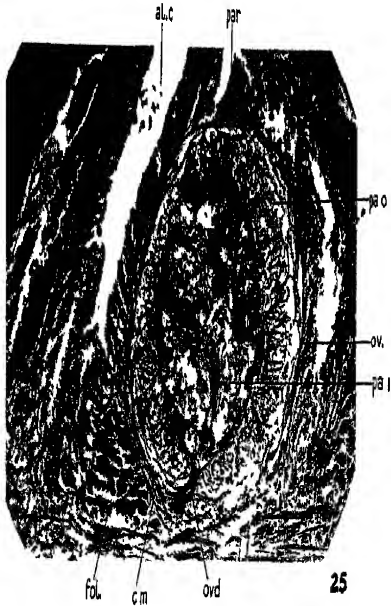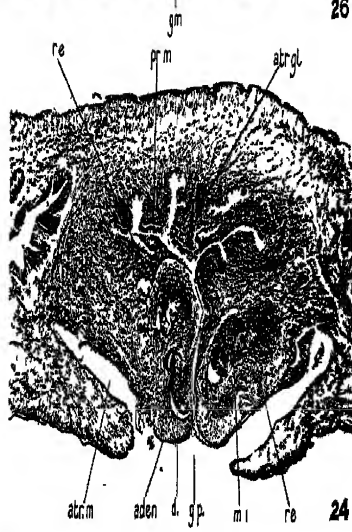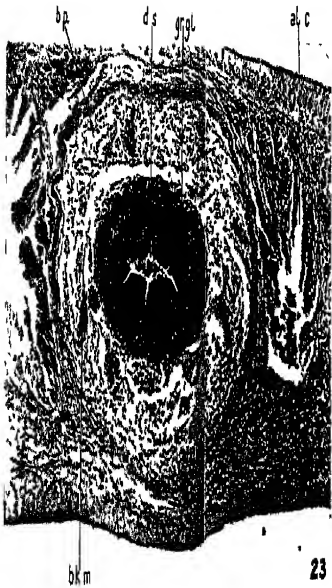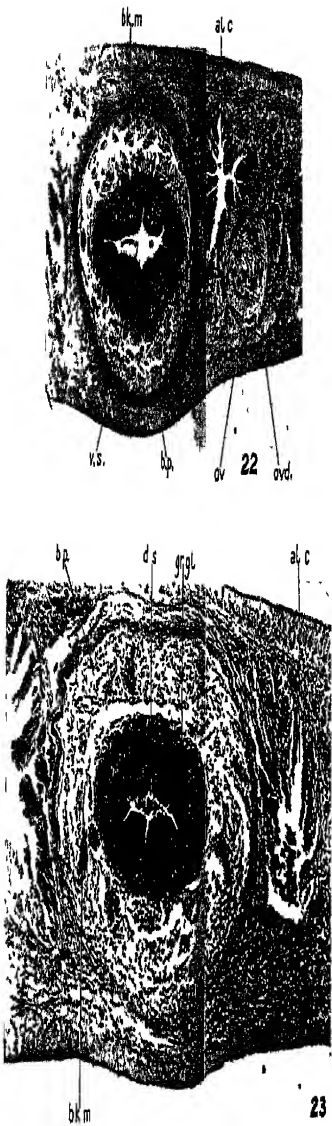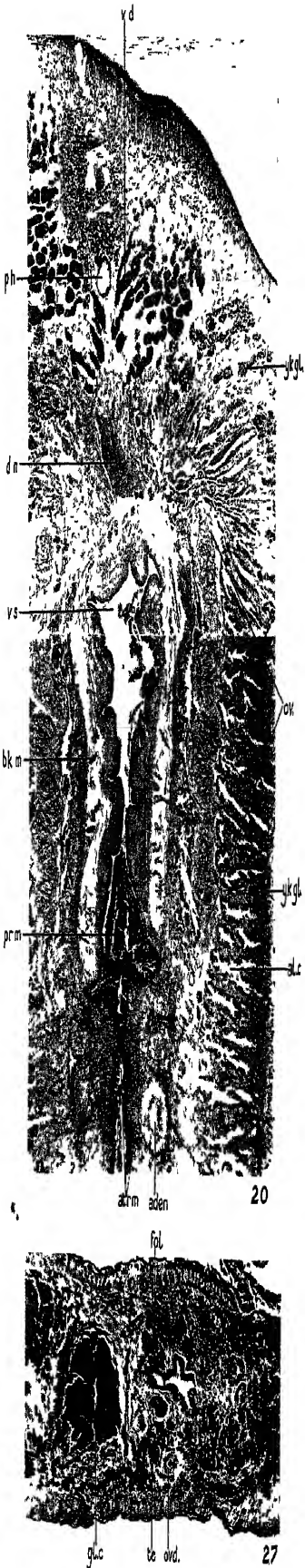
The Structure and Development of Wax Glands of Pseudococcus maritimus (Homoptera, Coccidae).

By

Priscilla Frew Pollister,

Department of Zoology, Columbia University

With Plates 17–20, and 3 Text-figures.

INTRODUCTION.

ONE of the striking features of the family Coccidae is a universal tendency to form extensive secretions of wax or wax-like substances on the surface of the body. This substance seems to serve a protective role, but in many instances the amount of it is so out of proportion to the necessity for this purpose that many entomologists have held that the exudation of this wax is more accurately to be regarded as a type of excretion of a material that is an inevitable by-product of the metabolism of animals feeding exclusively on plant juices.

The wax is secreted through pores in the cuticula, which are the openings from underlying glands. The present study is concerned with the distribution, minute structure, and development of these glands in one of the mealy-bugs, P s e u d o c o c c u s m a r i t i m u s Erhorn. There have been many descriptions of the structure and distribution of the external pores, and a few studies of the histology and development of the underlying glands. Many of these observations have been incidental to a work primarily concerned with the taxonomy of the group. The present study attempts to deal with the problem primarily from a histological and cytological point of view, since in these structures one finds some of the most elaborately differentiated types of glands.

The problem was suggested by Professor Franz Schrader, and I am indebted to him for advice and encouragement throughout the work.

MATERIAL AND METHODS.

The original stock used in this investigation was obtained from a commercial greenhouse in Falmouth, Massachusetts, where the mealy-bugs were growing on narcissus bulbs. A stock of these was kept on various bulbs during the winter months and on citrus fruits in the summer. The transfer to the fruit each summer was indispensable to the continued reproduction of the stock. Specimens from the stock were identified as Pseudococcus maritimus Erh. through the kindness of Doctor Harold Morrison of the Bureau of Entomology of the United States Department of Agriculture.

For the study of the external apertures of the glands preparations of the exoskeleton were made by the potash-magenta method as described by Dietz and Morrison, 1916. The fixation of material for sectioning is difficult because of the covering of wax, which must be softened and partially removed by touching the animals with a brush that has been dipped in absolute alcohol. Immediately after the application of the alcohol the animals are dropped into the fixing fluid, which must be hot. Good fixation apparently occurs when the alcohol has affected the wax just enough so that the fixing fluid will penetrate readily, but has not acted so long that the alcohol has reached the soft tissues of the animal. Many fixing fluids were tried and Allen's B 15 and Kahle's were found to give vastly superior results, so that the study was made entirely from material fixed in one or the other of these mixtures. The material was embedded in paraffin and sectioned at 6 or 10 micra, stained with iron haematoxylin, and in some cases counterstained with either eosin or light green.

OBSERVATIONS.

Types and Distribution of Glands.

In potash-magenta preparations of adult females of Pseudococcus maritimus one can see hundreds of gland-pores. Each pore is at the top of a small papilla projecting above the level of the surrounding cuticula. As seen from above these derm-pores are of three distinct types, to which, after Ferris

(1918), I shall refer as the triangular, cylindrical (or tubular), and the multilocular.

The triangular derm-pores show four openings, three elongated ones that form a triangle, and a fourth circular aperture within the triangular (Text-fig. 1 B). These pores are the smallest and
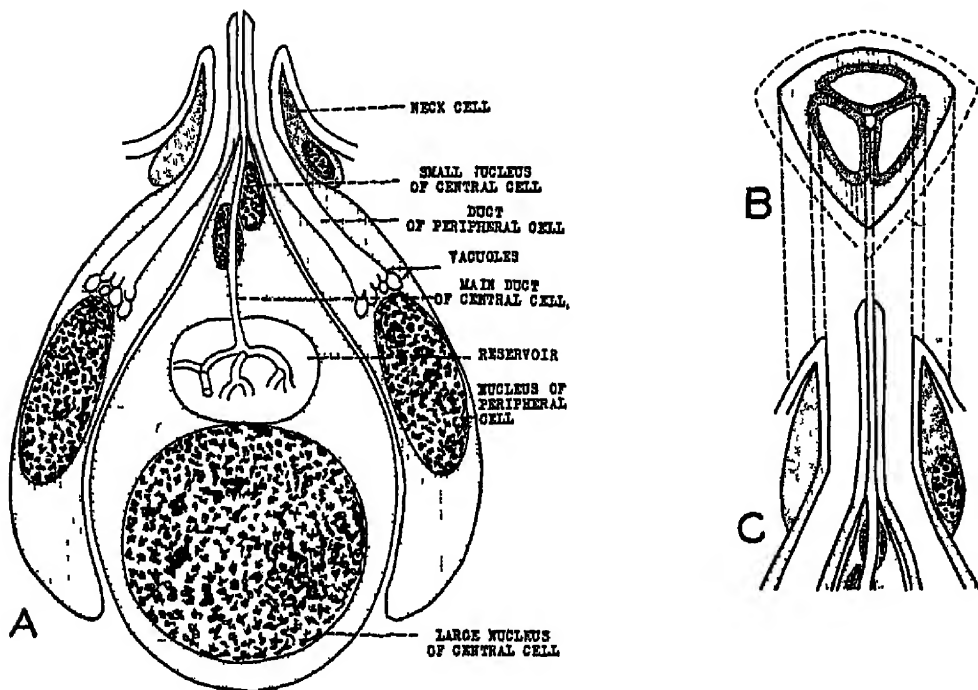
TEXT-FIG. 1.

A, diagrammatic sagittal section of triangular gland   B, pore of triangular gland as seen from above.   C, sagittal section of neck region of triangular gland drawn to the same scale as B.

most numerous. They are located on dorsal, ventral, and lateral surfaces of all segments. They are especially concentrated around the bases of the seventeen pairs of large lateral spines, with which they form the so-called cerarian complexes. They form small curled flakes of wax that are scattered over the surface of the body and are pushed outward along the large spines to form the long wax filaments projecting from their tips— a feature very characteristic of the genus Pseudococcus.

The tubular pores have a single large opening at the top of the papilla (Text-fig. 2). Like the first type, they are very numerous and are found on all surfaces. They are especially abundant on the ventro-lateral parts of the body, and within

each segment are more numerous at the anterior and posterior limits. These glands form large solid cylinders of wax.

The multilocular type of pore is much larger than either of the others and consists of a shallow cup at the top of a low papilla. The margin of the cup overhangs the cavity, within
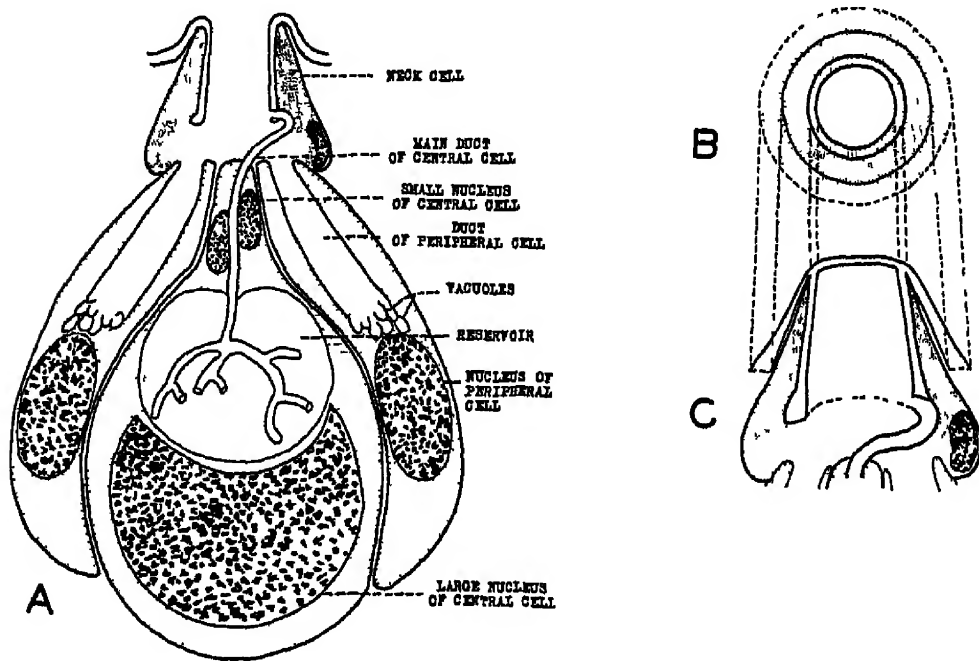
TEXT-FIG. 2.

A, diagrammatic sagittal section of tubular gland   B, pore of tubular
gland as seen from above   C, sagittal section of neck region of
tubular gland drawn to same scale as B

which is a peripheral circle of ten triangular openings surrounding a single central aperture. These pores are sometimes called circumgenital in allusion to the fact that they are especially concentrated in a ring around the vulva—although they are also found, in sparser number, on adjacent regions. The multilocular pores are much less numerous than the other types and are restricted to the anterior and posterior regions of the ventral surfaces of the last five segments. These glands form irregular masses of wax. In Pseudococcus citri Matheson (1928) seems to have made out a fibrillar texture to this wax, but such a finer structure is not identifiable in Pseudococcus maritimus.

Histological Structure of the Glands.

Triangular Glands.

The triangular type of gland is composed of five cells. As a whole the gland is roughly pear-shaped, with the narrow stem-like portion ending in a heavily chitinized triangular external pore at the top of a small papilla (fig. 13, Pl. 17). The *stem or neck of the gland extends from the base of the epidermis* (hypodermis) to the outer surface of the cuticula, while the body of the gland lies mainly below the epidermis (fig. 12, Pl. 17) The neck portion is formed from a single cell, the neck-cell or terminal cell (fig. 17, Pl. 17). The main body of the gland is made up of four elements—a large pear-shaped central cell partially surrounded by three more slender peripheral cells. The latter are fitted into deep grooves in the former (figs. 1 and 12, Pl. 17). The central cell contains three nuclei, a large spherical one in the distal end of the cell and two small ones at the proximal end nearer the epidermis (figs. 2–4, 5, 6, and 18, Pl. 17). Above the distal nucleus one finds a large, globular, clear space, the reservoir, and a system of tubes lined with chitin. This branched system of tubes leads into a single efferent duct which emerges from the top of the reservoir and traverses the narrow upper part of the central cell, from which it is continued through the centre of the neck-cell to terminate in the central aperture of the external pore (figs. 15, 16, and 17, Pl. 17). The three peripheral cells are uninucleate. Directly above the nucleus are clear vacuoles (fig. 21, Pl. 17) in the cytoplasm, and farther up these vacuoles run together to form a single space, the duct, which extends spirally through the upper end of each cell to the neck-cell (figs. 7–10, 12, 14, 18, and 19, Pl. 17). From the base of the neck-cell the ducts of the peripheral cells are continued as three tubes, which pursue a slightly spiral course through the neck-cell to end in the three peripheral openings that form the sides of the triangular external derm-pore of the gland (figs. 10, 11, and 13, Pl. 17). The structure of the triangular gland is indicated diagrammatically in Text-fig. 1 and Pl. 20.

Tubular Glands.

The tubular type of gland is composed of twelve cells, and, as a whole, is flask-shaped. The short neck portion is composed of a single cell that is located in the epidermis and contains a large central chitinous duct which opens to the exterior where it is continuous with the exoskeleton. The bulk of the gland is below the level of the epidermis and consists of ten peripheral cells completely surrounding a much larger central cell. The latter has essentially the same shape and internal structure as the central cell of the triangular gland. It contains three nuclei, one large and two small (figs. 55, 56, 57, and 64, Pl. 18). In the larger tubular glands the large distal nucleus of the central cell is shaped like a cup with one part of the rim higher than the rest (fig. 65, Pl. 18). The duct of the central cell emerges at its upper end and opens into the side of the large tube that is the main duct of the gland as a whole (figs. 63 and 65, Pl. 18). The internal structure of the peripheral cells is like that of the same cells of the triangular glands. Above the single nucleus are vacuoles which become confluent to form a triangular duct that fills the neck of the cell (figs. 51, 52, and 66, Pl. 18). The peripheral cells are fitted closely around the central cell and they occupy grooves in the cytoplasm of the latter. When there are spaces between the surrounding cells the cytoplasm of the central cell is pushed out to fill these spaces so that the gland as a whole has a smooth, globular outline (figs. 54–7, 61–2, Pl. 18).

As already stated, the neck of the tubular gland is composed of one cell, which is shaped like a truncated cone and which contains a chitinized tube which is of uniform diameter, except for a slight flare at the basal end. Here it is joined by the duct of the central glandular cell (fig. 63, Pl. 18). The triangular ducts of the peripheral cells do not open directly into the chitinized tube of the neck, but seem to end in a clear space within the base of the neck-cell and just below the bottom of the chitinized tube (fig. 63, Pl. 18). Because of this relationship to the peripheral cells, the base of the neck-cell, when seen in face view from above, appears to be a circular plate with ten peripheral triangular perforations (openings of the ducts of the

peripheral cells), and a single peripheral circular opening (duct of the central cell). The apices of the triangles face the slightly raised centre of the base of the neck-cell, which is the point where the central glandular cell abuts against this base. The nucleus of the neck-cell is found outside the ring of ducts and closely adjacent to the cell membrane (fig. 59, Pl. 18).

The complete structure of this complicated tubular type of gland can now be clearly understood if one examines figs. 45–53, Pl. 18, a series of slightly oblique sections from bottom to top of a single gland. These should at the same time be compared with the diagrammatic representations in Text-fig. 2 and Pl. 20. The central glandular cell and its large nucleus project below the rest of the gland and thus are cut in the first section (fig. 45, Pl. 18). In the next sections appear the reservoir of the central cell and, successively, the various peripheral cells and their nuclei (figs. 46–8, Pl. 18). In fig. 49, Pl. 18, the section is above the large nucleus of the central cell and shows the two small nuclei. The next section (fig. 50, Pl. 18) is through the chitinized tubes in the upper part of the reservoir, and it likewise shows, for the first time, sections of all ten peripheral glandular cells. Next (fig. 51, Pl. 18) one sees the single efferent duct of the reservoir and the ducts of the peripheral cells, the latter on the right. Fig. 52, Pl. 18, is of a section which, on the upper right, passes through the base of the neck-cell (compare with figs. 58 and 59, Pl. 18). Just to the left of the neck-cell is the efferent duct of the central cell entering the tubular main duct of the gland. The lower half of this oblique section passes through five peripheral cells, as they curve up over the central cell and converge to join the base of the neck-cell. Fig. 53, Pl. 18, shows the tubular pore of the gland on the surface of the cuticula.

Multilocular Glands.

The multilocular gland, like the tubular type, is formed from twelve cells, and is flask-shaped with a short, wide neck. The neck is formed from a single cell, that is penetrated by the necks of the eleven basal cells, and it ends in a highly characteristic external pore consisting of a single central aperture surrounded by ten regularly arranged peripheral openings—the whole overhung

by a scalloped border (figs. 40 and 42, Pl. 17) The general plan of this gland is much like that of the tubular type, from which it differs chiefly in the character of the external opening and in the relative sizes of the eleven glandular cells. For here the ten peripheral cells are very large and the central cell, by comparison, is dwarfed, is uninucleate, and is devoid of reservoir and complicated internal duct system. A cross-section through the lower part of the gland shows the peripheral cells as triangles, their rounded bases forming the outer circumference of the gland and their apices meeting in the centre (figs. 26, 27, and 33, Pl. 17). A higher cross-section, just below the neck region, discloses the small central cell wedged in between the apices of the peripheral triangles (figs. 29 and 34, Pl. 17). The ducts within the peripheral cells are like those in the other types of gland (figs. 28 and 29, Pl. 17). A section through the base of the neck-cell appears very similar to the base of the same cell of the tubular gland, with the circle of triangular peripheral ducts (figs. 24, 35, 39, and 44, Pl. 17). The nucleus of the neck-cell is seen on one side, fitted between the cell membrane and the peripheral ducts. The centre of the ring, however, in this gland is occupied by the upper part of the central cell, which projects into the neck-cell slightly. The nucleus of the neck-cell is seen in lateral view in figs. 40 and 41, Pl. 17. Unlike the situation in the tubular gland where the ten peripheral cells terminate at the base of the neck-cell—in the multilocular gland the basal glandular cells seem to continue up through the terminal cell and to open to the exterior independently of one another, each through a single member of the apertures in the pore (figs. 31, 36, and 37, Pl. 17).

Figs. 25–31, Pl 17, are from a series of sections, from bottom to top, of a multilocular gland, and should be compared with Text-fig 3 and Pl. 20. Figs. 25–7, Pl. 17, are through the bases and nuclei of the ten peripheral cells. Fig. 28, Pl. 17, shows, on the right, the supranuclear vacuolated zone of the peripheral cells. Fig. 29, Pl. 17, is from the next section, that passes through the nucleus of the central glandular cell and, on the right, through the base and nucleus of the neck-cell. Fig. 30, Pl. 17, is through the middle of the neck-cell, except on the left, where

several of the peripheral ducts are seen converging to the point where they enter the neck-cell. Fig. 31, Pl. 17, is from the uppermost section, an oblique view of the gland-pore and, on the left, the lower part of the neck-cell. A similar but less oblique series is illustrated in figs. 32–7, Pl. 17.
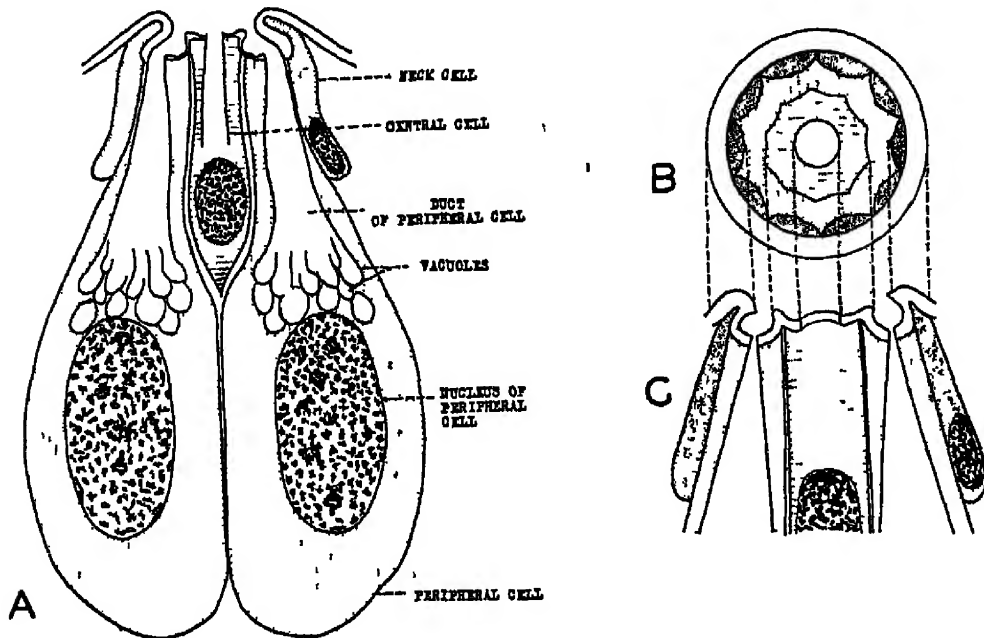
TEXT-FIG. 3.

A, diagrammatic sagittal section of multilocular gland. B, pore of multilocular gland as seen from above. C, sagittal section of neck region of multilocular gland drawn to same scale as B.

Development of the Glands.

It has been frequently pointed out that in the development of the female coccid there are three immature instars, which are distinguishable from one another by such characteristics as the number of antennal segments, of spines, and of gland-pores. The fourth stage, adult, of course, is very readily identified by the presence of the vaginal opening. In potash-magenta preparations these exoskeletal characters are very easily studied. The data in the Table are from such material. It will be observed that there are four types which are assumed to represent the four stages of development. The first group, with six antennal segments, a small number of spines, and only the triangular type of derm-pore, is the first instar. The tubular and multilocular

glands are not present anywhere on these specimens. A second group, representing the second instar, likewise has six antennal segments, but the number of spines and of triangular gland-pores is increased and the tubular type of pore appears. Other features found here, and not present in group one, are a thickened chitinized region around the last cerarian group of spines and a triangular area of thickened chitin on the dorsal surface of the last segment. These facts seem to justify considering the specimens in group two to represent the second instar. In a third group there are seven antennal segments; the number of triangular glands is approximately tripled; and the number of spines is typically doubled. A curiously inconsistent feature is a considerable reduction in the number of tubular glands. This group of specimens represents the third instar. The adults, group four, are, of course, unique in the possession of the vulva and the multilocular glands. They also show a further increase in the number of triangular and of tubular glands as well as spines.

Although counts were made only on the penultimate segment, the data are roughly representative of the whole body. Casual examination of any region shows that, with the exception of the tubular type between instars two and three, there is a progressive increase of the glands and spines at each step in development. Accordingly the details of the formation of the glands can be best studied in the post-embryonic period, especially between instars three and four when hundreds of each type are being formed.

Studies of ecdysis in insects (see especially Wigglesworth, 1933) have shown that the first stage in the process is the formation of a fluid between the cuticula and the epidermis, which separates these layers that were previously tightly adherent. The epidermis is thus freed and contracts to form a thicker epithelium of higher cells, and its constituent cells can then round up and divide independently of one another. Hence it is during this period that changes involving increase in the number of epidermal cells can occur. It seems that in Pseudococcus this early stage of ecdysis must be relatively brief, since in a great majority of the sectioned immature

TABLE.

Number of glands and spines of Pseudococcus maritimus found on the penultimate segment in the various instars and the adult female. The number of segments to the antennae, which is constant for a given instar and the adult female, is also shown.

	Segments of Antennae.	Triangular Glands.	Tubular Glands.	Multilocular Glands.	Spines.
1st Instar	6	6	0	0	14
	6	6	0	0	14
	6	6	0	0	14
	6	6	0	0	14
2nd Instar	6	27	11	0	19
	6	32	11	0	19
	6	41	14	0	19
	6	30	12	0	19
	6	30	12	0	19
	6	32	12	0	19
3rd Instar	7	108	3	0	38
	7	108	0	0	38
	7	97	1	0	38
	7	108	5	0	38
Adult	8	305	212	63	89
	8	253	195	58	74
	8	258	154	55	55
	8	303	186	68	77

specimens cuticula and epidermis seem continuous and there is no indication of cell division in the latter. In a few cases, however, a distinct space separates cuticula from epidermis, and in these specimens there are numerous mitotic divisions in the epidermis, and one finds various stages in the development of glands and spines.

Most of the mitotic figures are oriented with the axis of the spindle parallel to the plane of the surface of the epidermis, and these are obviously going to give rise to two cells that will be a part of the simple epithelial layer of the epidermis, increasing its area. Here and there, however, one finds mitotic figures with the spindle axis perpendicular to the epidermal layer, and it is obvious that from such a layer should arise one epithelial cell and one that is sub-epithelial, i.e. sub-epidermal (fig. 67,

Pl.19). Such figures are possible first stages of development of sub-epidermal structures, like the secretory cells of coccid glands, trichogen cells, and cenocytes.

Figs. 68 and 69, Pl. 19, show a condition that seems to be a somewhat later stage in gland development, where there has been an increase in number of sub-epidermal cells. Division of the cell continuous with the epidermis is contributing another cell to those below the epithelial layer. Fig. 73, Pl. 19, is evidently a stage slightly later than figs. 68 and 69, Pl. 19. It appears that before cell-multiplication[1] has been completed to attain the number characteristic of the mature functional gland, the uppermost cell, that is, within the epidermis, has differentiated the chitinized structures typical of the neck of the tubular gland. A similar condition for the multilocular gland is seen in figs. 72 and 81, Pl. 19, except that here it should be noted that the neck-cell has developed only the superficial opening of the gland.

It is well to digress somewhat at this point to emphasize the significance of the difference in development of these two types to the interpretations of their structure when fully formed. It will be recalled that it was stated (p. 133) that the ducts of the peripheral cells of the tubular gland terminate below the base of the neck-cell; while in the multilocular type (p. 134) these cells penetrate the neck-cell to open independently on the surface. This assumption concerning the latter type was preferred to the alternative one that the continuation of the peripheral cell-ducts through the neck-cell were differentiations within the substance of that cell, largely because of the situation shown in figs. 71, 72, and 81, Pl. 19—since here there is no trace of differentiated structure in the neck-cell below the level of the superficial cup, into which the eleven ducts open in the fully formed multilocular gland.

. Appearances like figs. 70, 75, and 77, Pl. 19, are obviously slightly later than figs. 72 and 73, Pl. 19. Here the sub-epidermal glandular rudiment is represented by a mass of cytoplasm containing many nuclei, of uniform size and smaller than those

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[1] Cell-multiplication is shown only by the number of nuclei since it is not possible to make out the cell boundaries in these early stages.

of earlier stages. Figs. 75, 77, and 78, Pl. 19, are all cross-sections of the sub-epidermal part of the rudiments of multilocular glands, and are of special interest because of the fact that each shows eleven nuclei. Since this is the number characteristic of the functional gland these figures must represent the end of the period of nuclear multiplication. (Note that in addition to the eleven nuclei of the potential glandular cells in figs. 75 and 77, Pl. 19, the neck nucleus is also seen at a slightly higher focal plane.) There should be a similar time when the sub-epidermal part of the rudiment of the developing tubular gland would show thirteen nuclei, but it is very difficult to count them when they are closely crowded together and I have not been able to determine one with more than the ten shown in fig. 70, Pl. 19.

The stage just described requires little further change to become the mature multilocular gland, i.e. the appearance of cell boundaries (fig. 78, Pl. 19), development of the secretory material in the cytoplasm of the cells, and the growth of the glandular cells through the neck-cell to reach the apertures of the derm-pore. In the case of the tubular gland an extensive differentiation of the central cell must occur. Figs. 76, 79, and 80, Pl. 19, show one later stage in the development of the tubular type. The large nucleus must be that destined to be located at the base of the central cell. There is no reservoir in the central cell, nor are there vacuoles in those at the periphery—so apparently this gland is not yet functioning.

I have not definitely identified stages in the development of the triangular type of gland. These glands have but five cells and it seems probable that development is much more rapid than in the other two types. The ducts of all four gland-cells seem to penetrate the neck-cell in the mature triangular gland, as do the cells of the multilocular gland, so that it seems likely that here also the neck-cell gives rise to at most only the outermost part of the gland-pore.

At an early stage of ecdysis, when the development of the glands is taking place, one finds numerous large irregularly shaped cells lying in the body cavity, immediately under the epidermis (figs. 71 and 74, Pl. 19). At first they are so numerous as to form almost a continuous layer. In the late stage of

ecdysis, after the glands and a new cuticula are formed, the number of these cells is much reduced. From their appearance and behaviour these cells are undoubtedly the structures variously known as oenocytes, coenocytes, ceridocytes, et als. Wigglesworth, working on Rhodnius, has very carefully described the relationship of these cells to the course of ecdysis, showing that in the later stages they become very much reduced in size, which leads him to believe them the source of the cuticular substance. Perhaps of more significance to the present study is the observation of Rogojanu, 1935, that in the aphid Erio soma the oenocytes are, in some parts of the body, restricted to the vicinity of the developing glands; where there is, in fact, a single oenocyte underlying each group of gland-cells that is a gland-field. Indeed, it is a remarkable fact that in Eriosoma Rogojanu finds that he can, as it were, predict the site of development of a gland by the presence of this cell below the undifferentiated epidermis. He suggests that one may regard the oenocytes of Eriosoma as serving a nutrient function for the developing gland-cells. It was possible to establish the above relationship in Eriosoma because the glands are few in number and restricted to very definite areas. In Pseudococcus the glands are so widely distributed that one cannot determine whether there is any definite correlation between the presence of oenocytes and glandular differentiation.

DISCUSSION.

The only extensive comparative study of the glands in Coccidae has been made on the family Margarodidae, a different group of coccids from that to which Pseudococcus belongs. In this group Morrison (1928) describes and figures the appearance of the derm-pores as seen in potash-magenta preparations of over a hundred species. It will be recalled that surface views of this sort of preparation of Pseudococcus maritimus show two categories of pores: one which has a simple circular outline (tubular, Text-fig. 1 B), and another with a marginal ring of openings surrounding a single central aperture (including the triangular and multilocular types, Text-figs. 2 B and 3 B).

The former is what Morrison calls the simple type, and he finds it confined to four genera. He applies the term multilocular in a general sense to the latter type of pore, which is found in every species of Margarodidae that he has examined. The derm-pores of the multilocular type show considerable variation within this general scheme of peripheral and central apertures. The number of marginal openings surrounding a single central aperture varies, in different species, and includes every number from two to seventeen. Furthermore, the number of central openings is variable, though within a narrower range. The most complicated derm-pore seems to be a type found in X y l o c o c c u s, where there are two concentric circles, each of twelve pores, and two central apertures. Morrison's work on this family is substantiated by numerous fragmentary observations of workers on other coccid groups. All the derm-pores of multicellular glands that have been described may be placed in one of Morrison's two categories, i.e. either simple or multilocular. The present study has demonstrated that as regards their sub-cuticular anatomy these two types are essentially identical, consisting of central and peripheral glandular cells. Hence one can make a general statement that this plan of histological structure is the rule for the wax-glands of Coccidae (see below, p. 142).

The histological structure of the wax-glands has hitherto been most carefully studied by Matheson, 1923, working on P s e u d o c o c c u s c i t r i, a form also earlier examined by Visart, 1894. As far as Matheson's observations go they agree very closely with the results of the present study on another member of the genus. In P s e u d o c o c c u s c i t r i are also found tubular, triangular, and multilocular glands that are distributed as in the adult P.s e u d o c o c c u s m a r i t i m u s, and each of these types is made up of a single central cell surrounded by a ring of marginal cells. Matheson, however, believes the number of the latter to be variable, but he agrees that a single aperture in the derm-pore is always the opening of a single peripheral glandular cell. In several other features Matheson's results differ from the present observations on P s e u d o c o c c u s m a r i t i m u s. He does not mention either the small nuclei or

the smaller ducts of the central cell, the spiral course of the peripheral ducts of the triangular gland, or the neck-cell.

In Icerya Murdock, 1928, has described the structure of a multilocular gland, which has two central openings in the pore. There is but one central cell, which discharges through the two apertures. This cell is only a little larger than the peripheral cells, and it has neither reservoir nor duct system —features in which it resembles the small central cell in the multilocular gland of Pseudococcus maritimus.

The work of Teodoro, 1911, on Pulvinaria is of interest to the present study chiefly because of the unique observation of the two small nuclei of the central cell. Otherwise his observations are almost certainly incomplete, since he apparently noted no peripheral cells.

In Saissetia Marshall, 1929, has described glands which evidently conform to the usual type, although cell boundaries could not be determined in his material. He mentions a large nucleus, above which is a 'vacuole' (reservoir?), 'accessory cells' (peripheral?), and other features that unmistakably suggest the histological characteristics of wax-glands of other Coccidae.

Very recently Rogojanu, 1935, has briefly described and figured in Orthezia a gland that is clearly very similar to the tubular type of Pseudococcus maritimus. Rogojanu also noted glandular areas containing but one type of cell, each cell opening independently on the outer surface. It seems to the author that these are more properly to be regarded as unicellular glands, and as such they constitute a unique exception to the usual method of wax production in Coccidae, resembling more closely the condition in Apis and some other insects. The case of Orthezia would probably repay a re-examination, more thorough than the study of Rogojanu seems to be.

The literature contains numerous incomplete observations showing more or less of the histological structure of the multicellular wax-glands of Coccidae, which need not be discussed in detail (e.g. see Putnam, 1878; List, 1886; Berlese, 1893; Moulton, 1907; Fullaway, 1910; Johnston, 1912; Childs, 1914). With the possible exception of Orthezia noted above, all of

the previous observations may be interpreted as consistent with the view that wax-glands of Coccidae are all modifications of one general scheme of histological structure in that they consist of two types of cells, distinguishable from one another by their orientation within the glandular mass—either in a central or a peripheral position. These cell types frequently show marked cytological differences as well. The peripheral cells often contain small scattered vacuoles which run together to form a large mass in the end of the cell nearest the pore. These are not found in the central cell. Instead, in those cases where its histological differentiation is marked it contains a single large vacuole or reservoir (presumably full of the special secretory product), and this is drained by a complicated chitinized duct system.

In view of the wide occurrence of the above type of gland in coccids the question naturally arises of the significance of these two types of cells in relation to the function of the wax-glands. In the present study the only possible method of approach to this problem is a comparative one. When one considers wax-producing glands in insects other than coccids it is found that, in most cases, as in bees and aphids, the glands contain but one cellular type, a relatively unspecialized cell much like the peripheral glandular units of the Coccidae. In bees the glands consist of a layer of these cells in the form of an extensive glandular field, on to the surface of which the wax exudes as an amorphous mass. This may be taken to indicate that the peripheral cells are the ones primarily concerned with the formation of wax in coccids; at least, it certainly shows that wax can be formed in the absence of the central cell type. Turning to other insect groups, one finds a wax-gland strikingly like those of coccids in an apparently distantly related group of Homoptera, the Fulgoridae. This was earlier described by Bugnion and Popoff, 1907, in Flata (Phromnia), and more recently Šulc, 1928, has described these structures in the same form and in other Fulgoridae.[1] Bugnion and Popoff considered the central

[1] Šulc's paper has not been consulted. It is written in Czech and is not readily obtainable. The reference to it is given in Weber's 'Lehrbuch der Entomologie', where two of his figures are reproduced. The suggestion concerning the function of the central cell is quoted in Rogojanu, 1935.

cell to be a nervous structure and Rogojanu (loc. cit.) has adopted this suggestion as the most likely for the gland of Orthezia. This view is manifestly absurd when applied to a structure like that of the central cell of the triangular and tubular glands of P s e u d o c o c c u s, and it seems to the writer that this cell must be glandular in function. When comparison between Fulgoridae and Coccidae is extended to the type of wax produced it is found that these two groups are alike, and differ from other insects, in secreting the wax in the form of accurately moulded filaments instead of an amorphous mass. This correlation of histological structure with function has been duly noted by Šulc, and he has suggested that the secretion of the central cell is not wax but a material that causes the wax elements produced by the peripheral cells to adhere to one another to form compound filaments having the dimensions of the pore. This hypothesis seems to the writer highly probable, and it is suggested that it be extended to apply to the central cells of the coccid wax-glands. In the present study there is a slight further support for Šulc's theory in the correlation of the degree of development of the central cell with the type of wax-form produced. It will be recalled that large wax filaments are produced by the tubular and triangular glands, in both of which there is a well-developed central cell. The multilocular type, however, has a very small central cell that is only slightly differentiated. Indeed, when it is compared with the central cell of the other two types it appears reasonable to consider it rudimentary and, in all probability, non-functional. In view of this condition it seems highly significant that the wax elements exuding from the apertures within the pore of this type of gland remain distinct, that is, do not fuse to form a compound product of specific shape, in marked contrast to the other two glandular types.

If Šulc's suggestion be assumed to be correct, then one may summarize the functioning of the typical coccid wax-gland as follows: the peripheral cells produce wax, which is squeezed out of the peripheral apertures in the form of filamentous structures. Simultaneously, from the central cell there comes a substance that will cause the filaments to adhere to one

another. In the tubular gland this mixture is squeezed within the tube and accurately moulded to form the uniform cylinder that has been often figured. In the case of the triangular type there is no mould, but the filaments must emerge in close contact with one another and in parallel spiral courses, and it is not difficult to conceive that under such circumstances they would adhere to form a definite compound structure, a large wax filament.

What is the nature of the material produced by the central cell that has this property of causing the filaments to adhere? The first thought, upon consideration of the properties of wax, is that this would be best accomplished by some volatile wax solvent. This leads to a comparative consideration of cells histologically similar to the central cell, a very specialized type. Cells with a similar chitinized internal duct system and reservoir (also sometimes chitinized) are also found in many Heteroptera, where they occur as unicellular glands that are generally believed to produce the substance responsible for the scent characteristic of many of these insects (see Weber, 1933). Many scents are volatile oils, and the most common wax solvents belong to this class of substances. To the extent that this comparison between cell types of rather remotely related insects seems acceptable as valid one can perhaps tentatively conclude that the central glandular cells of coccids produce a volatile oil which, by virtue of a solvent action on the filamentous wax products of the individual peripheral glandular cells, causes them to adhere to one another to form large compound filaments of wax.

In none of the previous studies of the histology of coccidian wax-glands has there been a description of the neck-cell. This is probably because of the paucity of work on the development of glands, since, as will be recalled, it was this aspect of the present study that led to the concept of a special cell responsible for formation of more or less of the external cuticular orifice of the gland. The neck-cell seems closely analogous to the so-called tormogen cell that occurs in relation to developing spines. This cell, as has been most clearly shown by Wigglesworth, 1933, is an individual of the epidermal layer, and in relation

to the outer layer of the tormogen cell there develops a cup-like depression of the cuticula. A trichogen cell lies immediately beneath the tormogen cell, and the developing spine from the former grows through the latter and emerges at the outer surface in the centre of the cuticular depression, so that the base of the mature spine is encircled by a groove. The resemblance of this course of development to the mode of origin of the relationship between the pore of the multilocular gland and the ducts of the glandular cells is obvious and striking.

The literature on the development of wax-glands of coccids is very meagre, represented by but two papers. Rogojanu, 1935, has studied the development of the glands of Orthezia, but since he was mainly concerned with cytological observations indicating the onset of function his results need not be reviewed here. His more significant observations on Eriosoma are considered on p. 140. The development of the glands of Saissetia was partially worked out by Marshall, 1929. Although he does not recognize a neck-cell of the gland he apparently observed the development of the tube before the differentiation of the glandular cells. His results are thus confirmatory of this unique aspect of the present concept of the morphology of the gland.

Summary.

The females of Pseudococcus maritimus have three types of multicellular wax-glands, one with a triangular external pore, another opening through a long tube, and a third with a multilocular aperture. The first two are widely distributed on all surfaces of the adult. The third is restricted to the ventral surfaces of the last five segments. This multilocular type is found only in the adult. The triangular glands are found at all stages and these structures progressively increase in number with each successive instar. The tubular type appears first in the second instar; the number is reduced in the third instar; and in the adult it is again increased to the largest number found at any stage.

The three glands are all modifications of one general plan of histological structure. The glandular elements are sub-epidermal

cells arranged in a ring of peripheral cells surrounding a single central cell. There are three peripheral cells in the triangular gland and ten in each of the others. The peripheral cells are uninucleate and contain vacuoles of secretory material. The central cell of the tubular and triangular glands has a large and two small nuclei and contains a large reservoir, from which a chitinized duct system leads to the gland-pore. The central cell of the multilocular gland is small and relatively undifferentiated. The author favours the view of Šulc that the wax is probably secreted by the peripheral cells, while the central cell secretes a substance that causes the wax filaments to adhere to form large cylinders.

The glands are developed by cell-multiplication from the epidermis at the time when it is freed from the cuticula at the beginning of ecdysis. After the initial period of cell-multiplication the first differentiation is the development of the external pore within the neck-cell. Later in the development of multilocular glands it is believed the glandular cells grow through the neck-cell to establish the functional relationship with the pore. It is suggested that this is analogous to the relationship between tormogen cell and trichogen cell in the development of a spine.

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EXPLANATION OF PLATES 17–20.

All the figures in Pls. 17, 18, and 19 were outlined with the camera lucida. In Pl. 20 are diagrams representing the author's conception of the three types of epidermal glands in the mature female of Pseudococcus maritimus. Pls. 17, 18, and 19 are drawn at a magnification of 2,300 diameters.

PLATE 17.

EXPLANATION OF FIGURES.

Figs. 1–21 are of triangular glands. Figs. 22–44 are of multilocular glands.

Figs. 1–7, 12, 14, and 18–21 were drawn from preparations fixed in Kahle's fluid. Figs. 8–11, 13, and 22–44 were drawn from preparations fixed in Allen's B–15 fluid. All of the above preparations were stained with haematoxylin and when a counterstain was used, either eosin or light green was employed. Fig. 15 was drawn from a total mount prepared by the potash-magenta technique.

Fig. 1.—Cross-section of a triangular gland.

Figs. 2–4.—Serial sections through a triangular type gland. Fig. 4 shows the two small nuclei of the central cell at the right and slightly below the reservoir which is indicated by a clear space. The upper right peripheral cell is cut above the nucleus and the duct is seen.

Figs. 5 and 6 show two different focal planes through a triangular gland: the large central nucleus, which is characteristic of the central cell, is clearly seen in fig. 5. Fig. 6 shows the position of the internal reservoir.

Fig. 7.—This section was cut at an oblique angle and shows the ducts of the peripheral cells in what is practically a longitudinal section.

Figs. 8 and 9.—Adjacent sections. Fig. 8 showing all three peripheral cells. Fig. 9 shows the duct of the central cell and the three peripheral ducts.

Figs. 10 and 11.—Two different focal planes of the same section. Fig. 10 shows the peripheral cells with their ducts and the central cell with a small part of the large distal nucleus and one of the two small nuclei. Fig. 11 shows at the upper right the nucleus of the neck-cell, the three ducts of the peripheral cells, the main duct of the central cell with one of the small nuclei of that cell.

Fig. 12.—Thick longitudinal section of a typical triangular gland showing all structural features.

Fig. 13.—Surface view, from above, of the external aperture of the triangular gland.

Fig. 14.—The lower focal plane of fig. 12.

Fig. 15.—Lateral view of the main duct of the central cell.

Fig. 16.—Internal reservoir of the central cell, showing the main duct of that cell running up to the chitinized external orifice of the gland.

Fig. 17.—The internal reservoir and duct system from a more deeply stained preparation. The main duct of the central cell is shown originating from the coalesced ducts in the internal reservoir, and running up through the cytoplasm of the central cell to the external chitinized orifice of the gland. The two small nuclei of the central cell are shown here one slightly below and at the left of the internal reservoir; the other slightly above and in the cytoplasm at the left of the main duct. Part of the neck-cell with its nucleus is seen at the left in this figure.

Fig. 18.—Longitudinal section through the central cell showing the distal nucleus, the internal reservoir, and the two small nuclei. Two peripheral cells are seen at the left.

Fig. 19.—A typical longitudinal section through a triangular gland.

Figs. 20 and 21.—Drawn especially to show the branches of the internal duct system of the central cell. The upper peripheral cell in fig. 21 shows the vacuoles.

Fig. 22.—Vertical section through pore and neck-cell of multilocular gland.

Figs. 23 and 24.—Adjacent transverse sections of upper part of a multilocular gland showing how the vacuoles of the peripheral cells (fig. 23) are continuous with the ducts.

Figs. 25–31.—Slightly oblique series of sections showing multilocular gland. (See text, p. 134.)

Figs. 32–7.—Another series of sections of a multilocular gland.

Figs. 38 and 39.—Like figs. 23 and 24. The nucleus of the neck-cell is seen at the left in fig. 39.

Fig. 40.—Longitudinal section of a multilocular gland showing the nucleus of the neck-cell at the right and in the upper centre the nucleus and duct of the central cell.

Fig. 41.—Longitudinal section of a multilocular gland showing the neck-cell.

Fig. 42.—Like fig. 40.

Figs. 43 and 44.—Two different levels through the same multilocular gland similar to figs. 23 and 24.

PLATE 18.

All the figures on this plate are of tubular glands.

Figs. 45–53 and 64–6 were taken from specimens fixed in Allen's modification of Bouin's fluid (B–15). All the other figures (54–63 and 65) were taken from preparations fixed in Kahle's fluid.

Figs. 45–53.—Serial sections cut at a slightly oblique angle through a tubular gland. (See text, p. 134.)

Fig. 54.—Cross-section showing the central cell, with reservoir and small nucleus, surrounded by the ten peripheral cells.

Figs. 55–7.—Sections cut at an oblique angle through a smaller tubular gland showing the large central nucleus and the two small nuclei characteristic of this cell. Figs. 55 and 56 are different focal planes of one section.

Fig. 58.—Oblique section through the upper part of a tubular gland.

Fig. 59.—Section through the base of neck-cell with the nucleus of that cell at the lower left. The main duct of the central cell is also seen in cross-section as a small circle adjacent to the nucleus. The central light area is not a duct but a section through the depressed centre of the base of the neck-cell.

Fig. 60.—Small tubular gland, lateral view, showing the characteristic arrangement of the nuclei. Cell membranes were not evident here.

Figs. 61 and 62.—Cross-sections through smaller tubular glands.

Fig. 63.—Longitudinal section showing external chitinous tubular duct of the neck-cell with the main duct of the central cell at the right.

Fig. 64.—Longitudinal section showing internal reservoir, ducts, and nuclei of central cell. The nucleus of the neck-cell is seen above.

Fig. 65.—Longitudinal section of tubular gland showing cup-shaped large nucleus of central cell partially enclosing the reservoir.

Fig. 66.—Longitudinal section of a small tubular gland.

<center>PLATE 19.</center>

Figs. 70, 73, 74, 76, 79, and 80 are of tubular glands. Figs. 71–2, 75–8, and 81 are of multilocular glands.

All the figures on this plate were drawn from preparations fixed in Kahle's fluid.

Fig. 67.—Section through the epidermis in an early stage of ecdysis: the cuticula, although not shown, is parallel to the top. Shows the elongated epidermal cells which have divided at right angles to the surface.

Figs. 68 and 69.—Early stages in the development of a gland rudiment by multiplication of an epidermal cell.

Fig. 70.—Young tubular gland showing duct in neck-cell and a ball of undifferentiated glandular cells below.

Fig. 71.—Rudiment of multilocular gland with two oenocytes below it.

Fig. 72.—Stage in development of multilocular gland comparable to fig. 70.

Fig. 73.—Young tubular gland. (More nuclei were present in the basal part of this gland than are actually represented here, as shown by adjacent section.)

Fig. 74.—Young tubular glands showing underlying oenocytes.

Figs. 75 and 77.—Sections through multilocular glands showing twelve nuclei. The darkly stained nuclei representing the glandular rudiment, the lightly stained one being the nucleus of the neck-cell.

Figs. 76, 79, and 80.—Later stages than fig. 70 of the tubular type gland showing enlargement of one nucleus.

Fig. 78.—Cross-section through basal glandular portion of multilocular

gland where indications of the individual cell membranes are noticeable.

Fig. 81.—Vertical section of young multilocular gland.

PLATE 20.

The three figures on this plate are diagrammatic representations of the three types of glands in Pseudococcus maritimus.

A. Triangular Gland. Two peripheral cells are shown on either side of the central cell: the third peripheral cell is assumed to be at the back and hence is not seen in the lower part of this diagram. The two peripheral cells shown are represented with portions removed to show the internal structure.

B. Tubular Gland. For the sake of clearness in this diagram two peripheral cells have been removed and two others are shown with the basal portions cut off. The basal membrane of the neck-cell shows the characteristic perforated plate formed where the central cell and the ten peripheral cells terminate in that membrane.

C. Multilocular Gland. Eight of the ten peripheral cells are shown, two having been removed to show the small central cell. The nuclei of the peripheral cells have been omitted.

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Neck cell

Internal duct

Small nuclei of central cell

Vacuoles

Efferent duct

Nucleus of Peripheral cell

Internal reservoir and duct system

A. TRIANGULAR GLAND

Large nucleus of central cell

Neck cell

Perforated basal membrane of neck cell

Small nuclei of central cell

Peripheral cell

Efferent duct

Internal reservoir and duct system

B. TUBULAR GLAND

Large nucleus of central cell

Neck cell

Central cell

Peripheral cell

C. MULTILOCULAR GLAND

On the Spinal Nerves of the Myxinoidea.

By

Edwin S. Goodrich, F.R.S.

With 4 Text-figures.

THE spinal nerve in the Gnathostomata is of mixed function, being formed by the junction of a mainly sensory dorsal root, provided with a ganglion, with a ventral motor root. The mixed nerve passes outwards posteriorly to the myomere supplied by its ventral root. Intersegmental arteries run dorsally from the dorsal aorta between the successive myomeres, and each artery passes up posteriorly to the spinal nerve. Segmental nerves and intersegmental arteries therefore alternate. These fundamental relations, illustrated in Text-fig. 1, are constant from fish to man, and can be seen in embryonic stages (Goodrich, 1930); but they may be modified in the adult.

In the Cyclostomata the conditions are different. It is well known that in the Petromyzontia the ventral root remains independent, and does not join the dorsal root to form a mixed spinal nerve. It passes directly to its myomere, while the dorsal root runs outwards posteriorly to this myomere. These relations are illustrated in Text-fig. 1.

The Myxinoidea, however, have mixed spinal nerves, since the dorsal root joins the ventral root anterior to it, apparently as in Gnathostomes.

The condition in Petromyzontia is almost certainly primitive, for in Amphioxus also the dorsal and the ventral roots remain separate. There is no doubt that Petromyzon is closely allied to Myxine, and that the Cyclostomata are a separate branch from the Craniate stem. In my book on Cyclostomes and Fishes (Goodrich, 1909) I adopted the suggestion of Koltzoff (1901), that the mixed nerve must have been acquired in the Myxinoidea independently of the Gnathostomata.

Searching for evidence on this point, the position of the

intersegmental artery, the importance of which was emphasized by Schauinsland (1906), has been studied. This artery occupies in P e t r o m y z o n the same position as in Gnathostomes relative
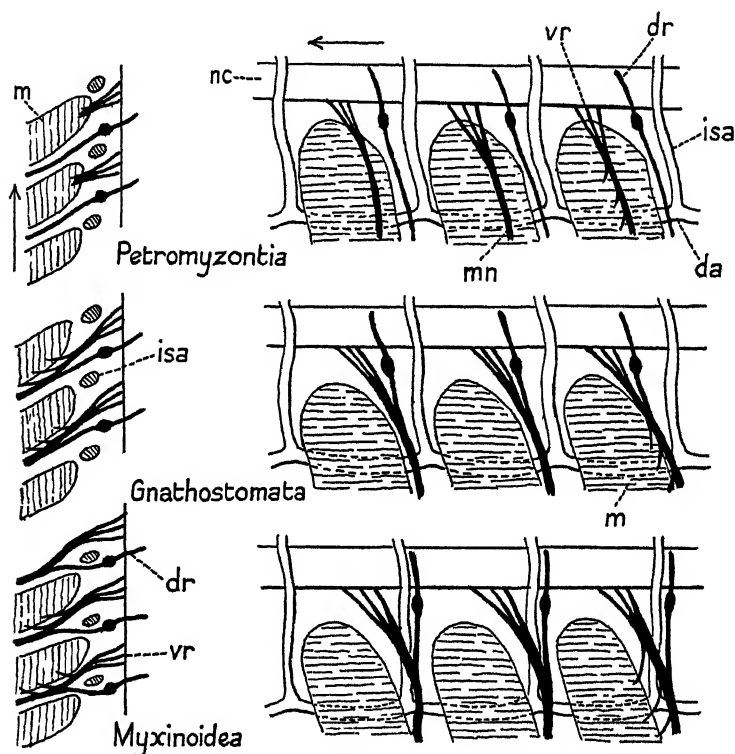
TEXT-FIG. 1.

On right diagrams showing relations of spinal nerves, myomeres, and intersegmental arteries. Seen from the left sides, with dorsal aorta passing behind myomeres. In P e t r o m y z o n t i a ventral nerve-roots are seen through myomeres. On left the same in longitudinal section.

to the dorsal and ventral nerve-roots, passing dorsalwards between the dorsal root of one segment and the ventral root of the segment next behind (Text-fig. 1). But in the adult M y x i n e it is seen on dissection to pass between the two roots which

combine to form one mixed nerve; that is to say, between the ventral root situated anteriorly and the dorsal root posteriorly. This is shown in Text-fig. 2 from a reconstruction Professor
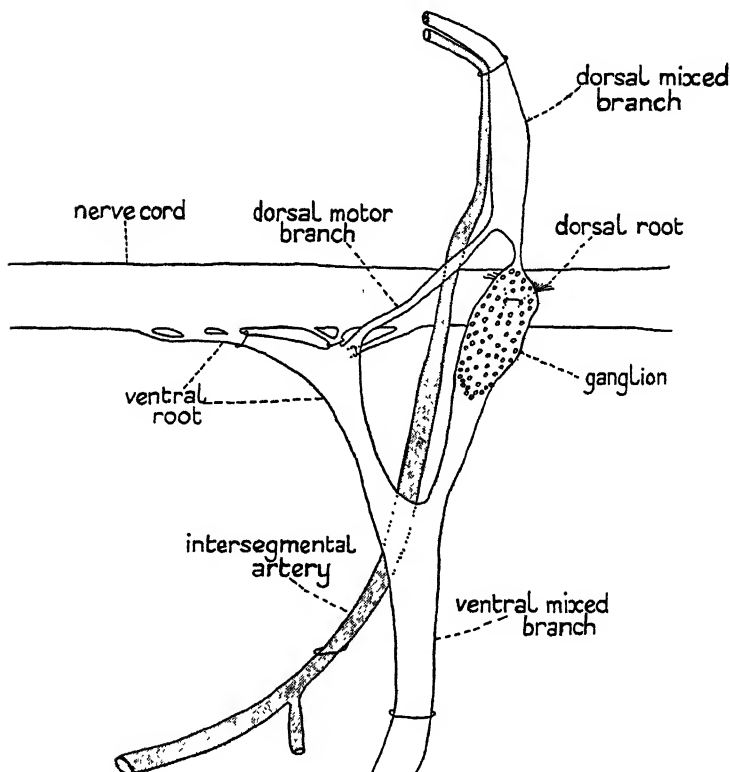
TEXT-FIG. 2.

Reconstruction, made by Professor Cole from transverse sections of adult Myxine glutinosa, of a spinal nerve and intersegmental artery. Left side view.

F. J. Cole was good enough to make at my request, and I have since confirmed it in various regions of the body from transverse sections kindly lent by Professor Cole.

To make sure that this adult vessel really represents the intersegmental artery it is necessary to study the embryo. I have to thank Professor G. C. Price for the loan of longitudinal

and transverse sections of embryos of Bdellostoma stouti, from the precious material collected by Dr. Bashford Dean. Examination of these sections shows quite clearly that the adult vessel is indeed directly derived from the embryonic inter-segmental artery (Text-figs. 3 and 4), which occupies its definitive
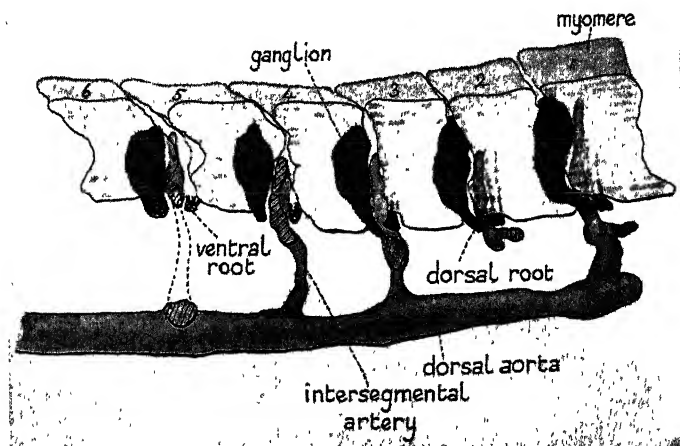
TEXT-FIG. 3.

Reconstruction, from longitudinal sections of an embryo Bdello-
stoma stouti, of a thick slice, slightly oblique. The inter-
segmental artery is seen between the ventral and dorsal roots.
Myomeres numbered from anterior end. Posterior trunk region.

and unique position from quite early stages even before the myotomes have been converted into myomeres, and before the two roots have combined to form the mixed nerve.

A comparison of the diagrams in Text-fig. 1 shows that the condition in Gnathostomes can easily be derived from the presumably more primitive condition in Petromyzontia on the supposition that in each segment the dorsal nerve root has simply joined the ventral root anterior to it. But there seems to be no plausible way of explaining the different relative position of the nerve roots and intersegmental artery in Myxinoids. For if we suppose the dorsal root to have joined the ventral root in front of it, the vessel does not pass between

them; and, if we suppose the dorsal root to have joined the ventral root behind it so as to embrace the vessel, then the ventral root is in the wrong place.

It may be objected that the position of arteries is not constant and may vary much in relation to surrounding structures,
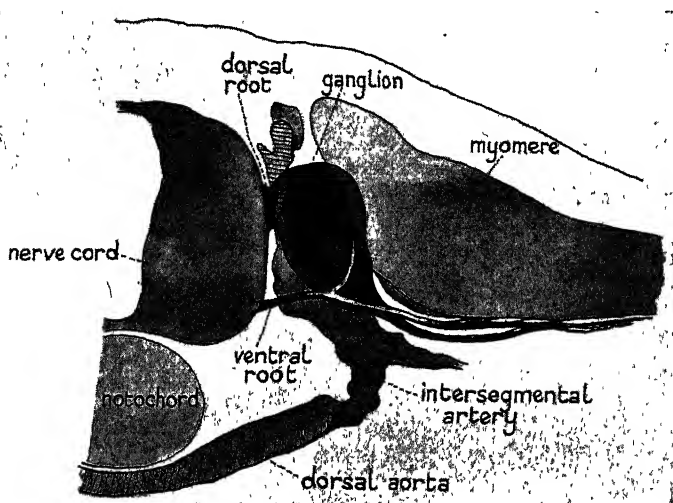
TEXT-FIG. 4.

Reconstruction, from transverse sections of an embryo B d e l l o -
s t o m a s t o u t i, showing the intersegmental artery between the
ventral and dorsal roots. Trunk region. Anterior view.

that adult arteries are derived from a general network of embryonic capillaries and may become developed later either on one side or the other of an intervening structure. But this is not what we find as a rule in vertebrates. Compare, for instance, the very constant relative position of the internal carotids to the trabeculae cranii, or of the stapedial artery to the stapes. When differences do arise their occurrence may generally be traced in development to the looping of the vessel or the disappearance of some part of the enclosing cartilage (Goodrich, 1930). I can find no clear evidence of such changes in Myxinoids, and at present the manner in which the differences

in the relative position of vessel and nerve has been brought about remains an unsolved morphological problem. Yet it may perhaps be not unreasonably held that the unique position occupied by the artery in Myxinoids supports the view that they have acquired a mixed spinal nerve independently of the Gnathostomes.

Department of Zoology and Comparative Anatomy,
University Museum, Oxford.

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The Embryonic Development of Calandra oryzae.

By

O. W. Tiegs, with assistance by Florence V. Murray,[1]

Zoology Department, University of Melbourne.

With Plates 21–26 and 19 Text-figures.

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A. Segmentation, p. 185. B. Stomodaeum and Proctodaeum, p. 191. C. Head and its Appendages, p. 192. D. Segmentation of the Insect Head, p. 196. E. Thorax and Abdomen, p. 199. F. Embryonic Membranes, p. 199.
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A. Early Development of Stomodaeum and Proctodaeum, p. 208. B. Mid-gut, p. 209. C. Discussion, p. 215.

[1] Much of the section-cutting and drawing has been the work of Miss Murray, whom I therefore wish to include as author of this paper. O.W.T.

M

INTRODUCTORY

THE following observations on the embryology of the rice weevil were intended, in the first place, as a contribution to the discussion on the formation of germ-layers in the insect embryo. An account of the post-embryonic development of this species having already been given (Murray and Tiegs, 1935), the observations were extended to cover the whole embryonic period; accordingly a reasonably 'complete' description of the development of this insect from egg to imago is now available.

Taken as evidence bearing on the phylogeny of the Insecta the embryology of a specialized form like Calandra is necessarily inferior in value to that of the more archaic types; but for the purely formal problems of insect development,

which alone have been considered here, it is the ready supply of technically suitable material that must decide the choice of subject, and as such Calandra has proved very satisfactory.

The extensive literature on insect embryology is remarkable for the diversity of opinion expressed even on matters of direct observation, important theoretical questions, notably a possible conflict with the germ-layer theory, being involved. It is therefore desirable that an intensive study be made of some readily procurable cosmopolitan species, rather than of numerous diverse forms which render comparison difficult. In the present case three relevant investigations are available—those of Tichomirow (1890) and of Inkmann (1933) on Calandra granaria, and Mansour's work on Calandra oryzae (1927).

Tichomirow's work is in the form of only a very brief note. Mansour's paper is concerned mainly with mid-gut development, but contains incidentally other observations of importance. Inkmann's work is devoted chiefly to the early phases of development, but mid-gut formation is also described.

The controversy over mid-gut development is well exemplified in these three papers, Tichomirow deriving it from the yolk-cells, Mansour from the proctodaeum and stomodaeum, while Inkmann alone supports the orthodox view of an endodermal origin.

MATERIAL AND METHODS

The weevils may readily be bred in captivity from wheat grains. The female lays her eggs singly in holes excavated with the rostrum in the grain, the hole being then sealed with a gluey secretion extruded from the ovipositor. The presence of this plug, easily recognized when the surface of the wheat grain is scanned under a low-power binocular lens, affords an easy means of detecting the egg. Infected grain is kept in an incubator at 25–6° C.

The egg is extracted by removing the hard shell of the wheat and breaking away as much of the endosperm as possible, without injury to the egg. Thus partially exposed it is immersed in saline, which softens the remaining endosperm, and enables the egg to be freed. Softening of the grain, by soaking in water

previous to exposure to the weevils, facilitates removal of the egg. Even with the utmost care, however, eggs, particularly if recently laid, are often broken during removal, for they are very fragile.

Of the various standard fixatives, those of Bouin, Gilson, Carl, and Bles give excellent results, particularly when used hot; with Heidenhain's 'Susa' mixture, which Inkmann recommends, the results were not as good, while Carnoy's fixative proved inferior. Mostly Carl's fixative has been employed, the eggs being immersed for 15 minutes in the fixative at 60° C. The fixation is usually very good, though occasionally the fixative fails to penetrate the chorion, while in some cases the egg bursts. The eggs are then transferred for a day to 70 per cent. alcohol, where the fragile chorion is partly or wholly removed with fine needles.

For staining whole embryos, by far the most satisfactory preparations have been made by use of the Feulgen method as applied recently by Schmuck and Metz (1931) and by Du Bois (1932) to whole eggs and embryos. With this method the chromatin is selectively stained, the embryo thus becoming sharply defined; the yolk, which remains uncoloured, may be counterstained with 'light green'. The method requires some practice, for overstaining easily occurs. Useful preparations can also be obtained with Auerbach's methyl green—acid fuchsin mixture. The embryos are cleared in clove oil and mounted in thin balsam, so that they can easily be rolled over under the cover-glass and examined from all angles.

For sections the celloidin-paraffin double-embedding method has been used, the ordinary paraffin procedure proving unsuitable for embryos. For the study of maturation and fertilization, however, paraffin embedding is quite adequate, for the yolk is not unduly hardened by xylol.

RATE OF DEVELOPMENT

To facilitate description the successive stages of development will be given in terms of the age of the embryo. It is not, of course, inferred that the rate of development is even approximately constant at fixed temperature; nor even that the relative

rate of development of different organs is the same for various embryos. In all cases the age assigned to a particular stage of development is the minimum age at which it has been found to appear.

The following table has been constructed for a temperature of 26° C.:

Age of Embryo.	Stage of Development.
Newly laid	Equatorial plate of first meiotic division (fig. 4, Pl. 21).
10 min.	First meiotic anaphase (fig. 5, Pl. 21).
25 min.	First polar body (fig. 9, Pl. 21).
65 min.	Second meiotic anaphase (fig. 14, Pl. 21).
80 min.	Just prior to fertilization (fig. 17, Pl. 21).
110 min.	7 cleavage-cells.
7 hr.	Peripheral distribution of cleavage-cells (Text-fig. 2 E).
7½ hr.	Cleavage-cells entering periplasm (Text-fig. 3 A).
12 hr.	Blastoderm; partitions between adjacent cells present but internal cell-wall unformed; secondary periplasm forming (fig. 30, Pl. 22).
17 hr.	Blastoderm as in Text-fig. 3 B.
20 hr.	Blastoderm; internal cell-walls fully developed; germ-cells invaginating (Text-fig. 3 D).
22–3 hr.	Lateral and median plates differentiating from blastoderm; embryonic membranes appearing (Text-fig. 4).
25 hr.	Dorsal flexure developing (Text-fig. 5).
27 hr.	Dorsal flexure further developed; head-lobes present; embryonic membranes do not yet enclose amniotic cavity (Text-fig. 7).
28 hr.	Appearance of mandibular segment (Text-fig. 8).
30 hr.	Maximum development of dorsal flexure; gnathal appendages appearing; embryonic membranes now enclose amniotic cavity; thorax segmenting; stomodaeal component of mid-gut arising.
38 hr.	Gnathal appendages prominent; segmentation extending along abdomen (Text-fig. 9).
42 hr.	Thoracic appendages present; abdomen segmented (Text-fig. 10).
48 hr.	Thoracic appendages prominent; somites with well-developed coelomic sacs; malpighian tubes and tracheal system appearing; proctodaeal component of mid-gut not developing yet.

Age of Embryo.	Stage of Development.
60 hr.	Shortening of germ-band beginning (Text-fig. 12); proctodaeal component of mid-gut present.
70 hr.	Shortening advanced (Text-fig. 13). Anterior and posterior components of mid-gut have met.
75 hr.	Shortening complete.
96 hr.	Larva emerges.

OBSERVATIONS

1. Maturation and Fertilization of the Egg

A. S t r u c t u r e o f t h e E g g.—The egg is semi-transparent, ovoidal, and rather more pointed at its anterior than posterior end; it measures on the average 0·6 mm. long, 0·27 mm. broad.

The chorion is uncoloured and unsculptured, and is very fragile.

Directly investing the egg protoplasm is a vitelline membrane, considerably thinner even than the chorion.

The egg-cytoplasm is concentrated mainly round the periphery of the egg as the periplasm (Keimhautblastem of Weismann), and two zones are usually distinguishable in it, an outer thinner and more eosinophil, and an inner granulated and more basophil layer. This stratification of the periplasm is recognizable in various figures on Plate 21, and recalls that already described for certain Lepidoptera (Schwangart, Huie, Eastham, Johannsen). The inner surface of the periplasm has a ragged appearance owing to the presence of the fine branching strands of protoplasm which spread inwards to form an anastomosing mesh supporting the entire yolk (fig. 23, Pl. 22; also various figures on Plate 21). The periplasm is of fairly uniform thickness except at the anterior pole of the egg, where it increases in quantity.

The yolk-grains are of variable size; occasional clear spaces between them suggest the presence of scattered oil-vacuoles.

The nucleus of the freshly laid egg lies within the periplasm, and is in the prophase of the first meiotic division. The diploid chromosome number is twelve (fig. 22, Pl. 21).

The orientation of the egg follows the usual rule, the morpho-

logically anterior end lying also anterior in the ovarian tube. There is no means of distinguishing dorsal from ventral surface.

B. Maturation.—The maturation phenomena that occur after laying are difficult to interpret without an examination of the unlaid egg. At first sight they seem to show post-reduction,
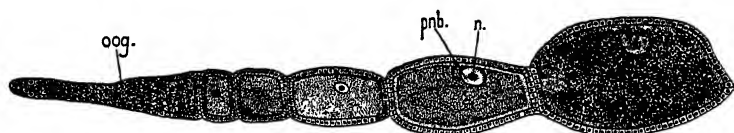
TEXT-FIG. 1.

Ovarian tube (semi-diagrammatic). The oldest egg shows break-down of perinuclear substance and its invasion by yolk; the younger eggs show the formation of this body, external to the true nucleus. *n.*, nucleus; *oog.*, oogonia; *pnb.*, perinuclear body.

and were described thus by Inkmann (1933) for Calandra granaria. Actually an obscured form of pre-reduction occurs.

An egg from the lower end of the ovarian tubes presents the following features (Text-fig. 1). The cytoplasm is already mainly concentrated at the periphery as the periplasm, from which arises the fine meshwork of anastomosing filaments that support the yolk in the interior of the egg. At the anterior pole the periplasm, at this stage, projects like a large plug into the yolk. Within the yolk lies a prominent spherical body with eosinophil granular protoplasm, and with a well-defined investing membrane (Text-fig. 1, *pnb.*). It is apt to be taken for the nucleus. It does not, however, give the specific Feulgen reaction; and moreover, as its development and subsequent history show, it is a specialized mass of cytoplasm with the true nucleus, invested by a peculiarly wrinkled sheath (figs. 1 and 2, Pl. 21), lying in its interior. In the oogonia, which form the tip of the ovarian tube, this perinuclear substance does not occur, and it is only with the gradual enlargement of the oocyte that it becomes apparent as a clear zone round the nucleus, becoming very prominent farther down the tubes as the oocytes increase in size. Finally while still in the ovarian tube the body disappears, this disappearance being initiated by a rupture of the membrane, adjacent yolk-grains then invading its substance

till eventually it ceases to be recognizable.[1] In the meantime the nucleus is moving towards the periphery of the egg, the wrinkled investing sheath gradually disappearing. By the time the egg has entered the vagina and is ready for laying the nucleus has passed into the periplasm (fig. 3, Pl. 21).

The early maturation phenomena occur during the passage of the oocytes along the ovarian tubes; it is not our purpose to describe these, since in regard to these particular stages the material is cytologically too unfavourable for such description to have any value. We begin the following account with the diplotene stage of the first meiotic division (from an egg in the last chamber of an ovarian tube). The appearance of the nucleus at this stage is shown in fig. 1, Pl. 21; the purpose of the illustration is to show the long filamentous form of the chromosomes at this period. The nucleus itself is encased in the shrunken sheath, external to which lies the perinuclear substance, of which a small quantity only is shown.

The chromosomes now begin to shorten, while at the same time the bivalent pairs again separate into their univalent constituents. This condition is shown in fig. 2, Pl. 21, from an oocyte still in the last ovarian chamber; the exconjugant

[1] This body, as Text-fig. 1 shows, is so readily taken for the nucleus, that further comments are desirable. It may be suspected that the body is a nucleus in the germinal vesicle stage, with diffuse chromosomes, the central body being a chromatin-nucleolus. But this is not the case, for as fig. 1, Pl. 21, shows, the chromosomes themselves are confined within the central body, and undergo conjugation there. A chromatin-nucleolus stage, intervening between conjugation and polar body formation does not, indeed, seem to occur in this species.

A review of the literature bearing on this point is beyond the scope of the present paper. That the condition is not peculiar to Calandra may be inferred from Wheeler's description (1889) for Blatta and Doryphora, in which a similar body, regarded however as a nucleus, was observed to fragment shortly before maturation, in a manner recalling the above account for Calandra; while Henking (1887), using a defective technique in vogue at the time, concluded that in a species of phalangid investigated by him the nucleus even completely disappeared before polar body formation; cf. also Ayres' (1884) account for Oecanthus.

Possibly the body is related to the 'pallial substance' that has been described as investing the nucleus in the eggs of certain arthropods. (See Wilson's 'Cell', 1925, p. 341.)

chromosomes have contracted into very short thick bodies, 11 of the 12 being visible in the section, most of these, moreover, appearing almost split into two. Fig. 3, Pl. 21, shows the condition of the chromosomes from an egg removed from the vagina; the nucleus has entered the periplasm, the investing sheath has disappeared, and 12 chromosomes, some partially split into two, are distinguishable. The splitting is in preparation for a precocious separation that will occur in the first meiotic anaphase.

In the newly laid egg the chromosomes have entered the equatorial-plate stage of the first meiotic division (to obtain this stage the egg must be extracted from the wheat grain as quickly as possible after laying). The twelve univalent chromosomes have again congregated into six pairs; each chromosome is now rather more rod-shaped than in the previously described stage, the splitting not being always recognizable. For those chromosomes where the splitting is visible there is a definite indication of tetrad formation (fig. 4, Pl. 21).

About 10 minutes after laying, the egg is in the first meiotic anaphase (fig. 5, Pl. 21), the chromosomes separating into two lots of six, in many of which the tendency to precocious splitting is recognizable. Fig. 6, Pl. 21, shows the condition at late anaphase, in which the polar body is just beginning to protrude; actual division of some of the chromosomes has now occurred, and as far as it is possible to count them, there are now nine chromosomes (chromatids) in the polar body and eight at the opposite end of the spindle, an accurate count being however difficult because it is not possible to distinguish with certainty between partially and fully divided chromosomes. In the example shown in fig. 7, Pl. 21, there are nine chromatids in the polar body, nine or ten at the oocyte end, and the full diploid number is apparently in process of formation, since a few chromosomes at either pole of the spindle are evidently in a state of incomplete division.

To give objective evidence of the complete separation of the chromatids to yield the diploid number at the end of the first meiotic division, the photograph shown in fig. 8, Pl. 21, from a stage a little in advance of that shown in the previous figure, is offered. It is from a longitudinally cut egg, so that the polar body

is not present in this, but in the adjacent section; the chromatids by good fortune are all within the plane of focus, eleven being recognizable, with one of them as yet incompletely divided.

It appears then that by the end of the first meiotic division, although reduction has already occurred, the diploid number of chromosome elements is present, this being due to a precocious separation of chromatids late in the anaphase.

In the meantime the polar body has begun to protrude more prominently from the surface and eventually becomes almost completely constricted off; it lies at this stage in a depression on the surface of the egg (fig. 9, Pl. 21); its cytoplasm is always markedly eosinophil and the chromosomes do not become enclosed within a definite nuclear membrane.

In the oocyte nucleus, on the other hand, a nuclear membrane is produced. The twelve chromosomes, however, do not clump together but proceed forthwith to the second meiotic division. From now onwards there is a considerable accumulation of periplasm at the site of the oocyte nucleus, the latter moving away from the surface of the egg and projecting prominently into the yolk (fig. 17, Pl. 21).

The second meiotic division, which begins less than an hour after laying, first becomes recognizable by a tendency of the chromatids of the oocyte nucleus to unite again in pairs (fig. 10, Pl. 21); and the pairing may become so intimate that the two components of the pairs are hardly recognizable (fig. 11, Pl. 21). This recoupling of the chromosomes is noteworthy; it has been referred to as preceding also the first meiotic division.[1]

At the equatorial plate stage (fig. 12, Pl. 21) spindle-fibres have become more distinct. The anaphase is shown in fig. 13, Pl. 21; six chromosomes are moving to opposite poles of the spindle; the nuclear membrane is still intact and the spindle is

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[1] While reference to the extensive bibliography on maturation is beyond the scope of the present paper this peculiar phenomenon needs further comment. It was first observed by Agar (1911) in Lepidosiren, and has since been recorded by Hogben (1920) for parasitic Hymenoptera—in both cases for the first meiotic division only. The present case is therefore of unusual interest in that it precedes both meioses, the diploid number of chromosomes having been restored by precocious separation of the uni- valents late in the first anaphase.

completely intranuclear. Disappearance of the nuclear membrane begins in late anaphase (fig. 14, Pl. 21; 65 minutes after laying), and in the early telophase is no longer recognizable (fig. 15, Pl. 21). At this period the chromosomes have clumped together and can no longer be individually distinguished. In the late telophase the clumping is still denser. Finally, each nucleus enters the resting condition (fig. 16, Pl. 21). It is noteworthy that in this respect the nucleus of the second polar body differs from that of the first; unlike the first, moreover, it remains throughout in the periplasm, there being no protrusion of a second polar body beyond the surface of the egg.

Division of the first polar body does not occur; the precocious division of its chromosomes in the first meiotic anaphase has already been noted.

C. Degeneration of Polar Bodies.—Reabsorption of the first polar body into the periplasm begins even before the second polar body forms, several stages being seen in figs. 12, 14, 16, 20, Pl. 21. In some eggs it is completed even before fertilization (fig. 16, Pl. 21); on the other hand, we have an egg as late as the eleven-cell stage when it is still in progress.

Degeneration of the nuclei begins usually at about the time of fertilization. In the second polar body nucleus the first indication is a marked swelling; its chromatin then becomes resolved again into chromosomes, distinguishable now from those of the first body by their much greater length (fig. 20, Pl. 21). Although they sometimes appear quite normal, yet more frequently they already show a tendency to fragment and clump together, and then stain very deeply. The nuclear membrane now disappears (fig. 21, Pl. 21).

Fragmentation of the chromosomes proceeds during the early phases of cleavage. We have one egg at the eight-cell stage in which they have almost vanished, while in another at the fifty-cell stage they are still recognizable. During the process of degeneration the two lots remain for a while separate, those of the second polar body being often distinguishable, by their greater length, from those of the first (fig. 21, Pl. 21); but later as the fragmentation proceeds they mingle, become scattered as fine globules in the periplasm, and eventually disappear.

D. Fertilization (nuclear fusion).—In most of the eggs polyspermy has been observed. The sperms apparently enter at the anterior end where the periplasm is unusually thick; according to Inkmann a micropyle occurs here (Calandra granaria). They then migrate deeper into the egg and are easily detected by the prominent mass of cytoplasm within which they lie (fig. 14, Pl. 21). They are confined to the anterior third of the egg and may occur up to five in number. The sperm-head at this period is usually a very deeply staining rod; the adjacent cytoplasm is very prominent, crescentic in form, the central pale axis probably lodging the sperm-tail (fig. 23, Pl. 22).

Less than an hour after laying the sperm-heads have become converted into rounded deeply staining hyaline bodies, in which chromosomes however are not yet visible. Towards the completion of the second maturation division they have begun to assume the appearance of a resting nucleus, though still rather hyaline. By the time the female pro-nucleus has formed the male nuclei are fully developed (fig. 16, Pl. 21).

The female pro-nucleus, already at late telophase, begins to move in the direction of the male pro-nuclei. Soon, apparently, the movement becomes a very active one, for a long trail of cytoplasm is drawn out by the female pro-nucleus as it passes through the yolk (fig. 16, Pl. 21). Judging by the track of cytoplasm, the female pro-nucleus moves almost in a straight line. Unlike the male it is invested by only the smallest quantity of cytoplasm; indeed, it is sometimes reduced to an imperceptible quantity, and were it not for the trail of cytoplasm that it leaves behind, it would be difficult to locate among the yolk grains. It resembles the male pro-nucleus in appearance, but is rather larger.

The female pro-nucleus now becomes closely applied to the male, which has in the meantime enlarged; the nuclear material is again resolved into its chromosomes, which are now long delicate threads. The condensation of cytoplasm around the pairing nuclei is very prominent (figs. 17, 18, Pl. 21). The nuclei then fuse, and the chromosomes intermingle (fig. 19, Pl. 21). A resting nucleus does not seem to be reconstituted; on the contrary the zygote-nucleus appears to divide at once,

and rapidly, for of many eggs examined at this period (80–100 minutes after laying) none were obtained showing any transition between the zygote-nucleus and the two cleavage-cell stage.

The supernumerary male pro-nuclei degenerate, and indeed, surprisingly soon. We have one preparation of a partially degenerate pro-nucleus surviving the fertilization; in all other eggs they have passed beyond recognition.

2. CLEAVAGE

In Calandra successive cleavages are, from the beginning, usually distinguished by a complete lack of synchronization; we have, for example, one egg with three cells, one only of which is dividing, another with eight cells, of which only two are in division, while in one egg with thirty-four cells only ten are in that condition. For insects this seems to be unusual, strict synchronization of mitoses being reported for various species— Hydrophilus (Heider, 1889), Donacia (Hirschler, 1909), Musca (Blochmann, 1886), Blatta and Doryphora (Wheeler, 1889), Eudemis (Huie, 1918), Pieris (Eastham, 1927), Ephestia being remarkable in that it extends to the 512-cell stage (Sehl, 1931). On the other hand, Platner (1888) finds a lack of synchronization in Liparis.

Amitosis of cleavage-cells, as reported by Wheeler for Blatta and by Strindberg for Eutermes, has not been seen.

From the beginning of cleavage the daughter-cells migrate apart, becoming gradually spread through the yolk. Here they are seen as conspicuous clumps of protoplasm, local islands in the protoplasmic mesh that pervades the whole interior of the egg, and which connects the cleavage-cells with one another, and with the periplasm, into one large syncytium. Direct connexion can, of course, only be displayed for closely adjacent cells (fig. 25, Pl. 22).

The early cleavage-cells are confined to the anterior part of the egg (Text-fig. 2 A–C). By about the 36-cell stage they have spread also into the hinder part, and show already at this period a tendency to place themselves concentrically to the periplasm (text-fig. 2 D). As cleavage proceeds this condition becomes better defined until, at about the sixth to seventh hour, the
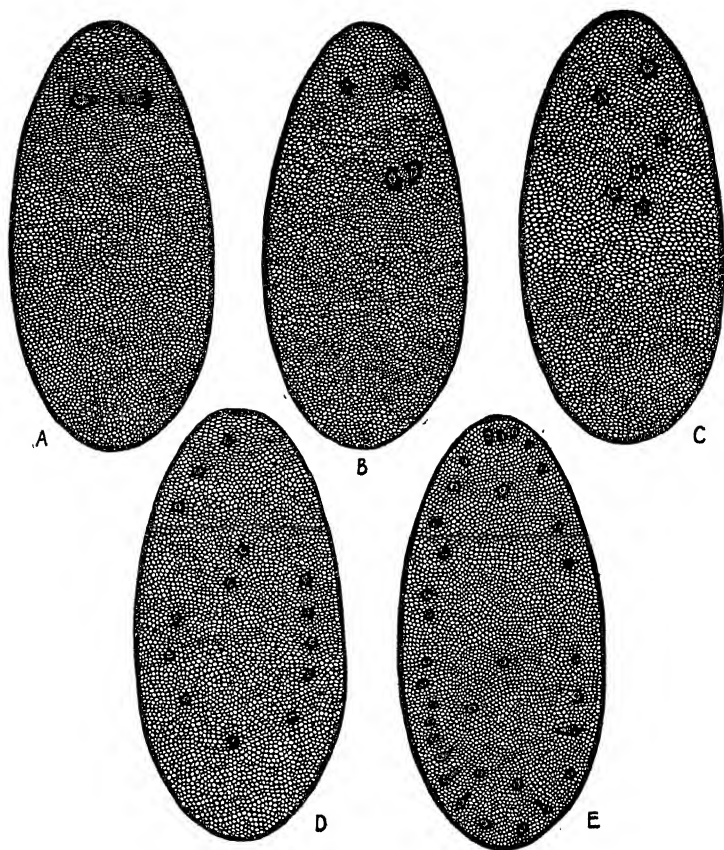
TEXT-FIG. 2.

Cleavage. Only such cells as are present in a thick median section
    along the egg are shown. A, 2-cell stage; B, 4-cell stage; C, 6-cell
    stage; D, 36-cell stage; E, about 150-cell stage. Segregation of yolk
    cells and blastoderm-cells apparent in D and E. Note 'comet-
    cells' in E.

well-known peripheral distribution of cleavage-cells is attained,
with a few cells remaining in the interior as the future yolk-
cells (Text-fig. 2 E).

How is this migration brought about? It is generally attri-
buted to some form of amoeboid movement on the part of the
cleavage cells. Eastham (1927) suggests, however, from his

observations on Pieris, that a centrifugal streaming of cytoplasm occurs, and that this plays a prominent part in drawing the cells to the periphery. In support of this is the fact that the cytoplasmic mesh inside the line of advancing cleavage-cells is much less conspicuous than outside that line. This has been noted also for other insects—Lasiocampa (Schwartze, 1899), Chalicodoma and Anthophora (Carrière and Bürger, 1897), Apis (Nelson, 1915), but is usually interpreted as an incorporation of the cytoplasm into the enlarging mass of cleavage-cells. Sehl (1931), however, has observed a definite streaming of the internal cytoplasm towards the ventral periplasm shortly after the beginning of cleavage (Ephestia). But for Calandra this explanation fails. A centrifugal streaming does, indeed, occur later in immediate connexion with blastoderm formation (q.v.), but prior to this there is no evidence for diminution of the internal cytoplasm. The frequent occurrence of the peculiar well-known 'comet-cells' seems, on the contrary, to show that the power of movement resides within the cells themselves. For example, fig. 24, Pl. 22, shows the separation that is effected at the end of the first cleavage; the advancing end blunt, with long following trail of cytoplasm, evidently suggests a cell forcing its way through the yolk.

Do the cleavage-cells spread at random through the yolk, or does the cleavage proceed according to some regular pattern as in other animals? It seems impossible to obtain microscopical evidence on this point, the spindles of dividing cells showing no recognizable orientation in respect to the egg as a whole. It should be observed that in the collembolan Tomocerus the yolk itself undergoes cleavage (Uzel, 1898), movement of cleavage-nuclei being therefore definitely circumscribed. On this point the genetic evidence seems decisive: the theory of Morgan and Bridges on the origin of gynandromorphs in Drosophila implying an absence of indiscriminate intermingling of the cleavage-cells, while the preponderance of bilateral gynandromorphs in that insect shows that the plane of initial cleavage is usually along the axis of bilateral symmetry of the imago. In the few eggs that we have at the two-cells stage in Calandra the cleavage-cells have undoubtedly

passed into opposite halves of the egg (Text-fig. 2 A; fig. 24, Pl. 22), but beyond this no definite cleavage pattern is microscopically recognizable.

3. FORMATION OF BLASTODERM AND YOLK-CELLS

A. The Blastoderm.—This forms, at the earliest, seven to eight hours after laying, 100–150 cleavage-cells being present at the time, comprising a peripheral zone of future blastoderm cells, numbering about 90 to 130, with between 15 to 30 yolk-cells scattered in the interior (Text-fig. 2 B).

Entrance of the cleavage-cells into the periplasm is not, as with most insects, confined to a particular region, but occurs uniformly over the whole surface (text-fig. 3 A).

As observed also for other insects—Neophylax (Patten), Chalicodoma and Anthophora (Carrière and Bürger), Apis (Nelson), Eudemis (Huie), Pieris (Eastham)—the mesh of cytoplasm pervading the yolk now becomes reduced to an almost imperceptible amount owing to a centrifugal flow into the periplasm, which draws in the cleavage-nuclei with it. 'Comet-cells' in fact no longer occur, many of the cells showing, if anything, a tendency to flatten against the periplasm (fig. 27, Pl. 22).

With the entrance of the cleavage-cells into the periplasm the cytoplasms of the two merge into one. Yolk-grains are apt to be carried in with the cells.

There is some uncertainty as to the behaviour of the peripheral cleavage-cells just prior to their entering the periplasm. In Calandra granaria, according to Inkmann, the cells pass into a phase of mitosis with radially directed spindles, the outer daughter-nuclei entering the periplasm, while those at the inner end of the spindle remain as yolk-nuclei. This would be a striking observation if correct; we are, however, unable to confirm it. A stage comparable to Inkmann's is shown in fig. 26, Pl. 22 (from a 7-hour egg, with 146 cleavage-cells including 20 yolk-cells); it is evident that in only one of the four nuclei drawn is the spindle radial—tangential spindles indeed predominate in the preparation. Inkmann's conclusion may be tested by determining the relative rate of increase of yolk-

and blastoderm-nuclei at this period. In the egg from which fig. 26, Pl. 22, is drawn there are 20 yolk-nuclei, 8 being in mitosis. Yet in two rather older eggs, with blastoderm already formed, and containing respectively 430 and 700 cells, the number of yolk-nuclei is not more than 45 and 55. The products of cleavage of the peripheral cells have therefore entered the periplasm and not the yolk.

Examination of a large series of preparations at this stage has shown that division of the peripheral cells before entering the periplasm is unusual. More commonly the asters, which are very prominent at this period, only become recognizable at the time of entrance. An example of this is shown in fig. 27, Pl. 22, from an egg with 15 yolk-cells and 90 peripheral cells, while in fig. 28, Pl. 22 (30 yolk-cells, 95 blastoderm-cells), with blastoderm already formed, mitosis is only beginning. Whether the precocious division is completed before entrance into the periplasm, or whether dividing cells are drawn in, is uncertain. There is evidence that the latter is the case; for we have one preparation (30 yolk-cells, 121 peripheral cells) in which the latter, mostly in mitosis, are some within, others without the periplasm—40 have entered (30 of them germ-cells), 61 are still outside, and the remaining 20 are transitional.

Within the newly formed blastoderm it is usual to find almost every cell in mitosis, the spindles being roughly tangential (fig. 29, Pl. 22). The surface of the blastoderm is very irregular owing to protrusion of individual cells. Cell-walls are not yet present.

The completely developed blastoderm is not seen till about the end of the first day. The visible changes that occur during the interval involve (i) a great increase in the number of its cells, the rather loose irregular layer of large flattened or cubical cells giving place to a compact epithelium of narrow columnar cells; (ii) the formation of cell-walls.

At about the fifteenth hour the blastoderm has the following appearance (fig. 30, Pl. 22): the cells have considerably increased in number and decreased in size, and lateral cell-walls have begun to appear, though there is as yet no indication of the internal cell-walls. Internal to the row of nuclei the protoplasm

of the cells is usually rather spongy, and this merges into a gradually thickening zone of exceptionally spongy cytoplasm adjacent to the yolk—the 'secondary periplasm' of Weismann. The latter arises apparently from a local synthesis of protoplasm, and not from a centrifugal flow from the interior of the egg, as Heider (1889) states for Hydrophilus, for the cytoplasm within the egg has already been reduced to an almost negligible quantity.

A later stage of development is shown in fig. 31, Pl. 22. The cells have further increased in number, and are becoming more distinctly delimited from the underlying periplasm, while at the same time an indication of the internal cell-wall appears within the reticular mesh of the secondary periplasm. This cell-wall formation advances, in the entire blastoderm, from behind forwards, the section being drawn from the transitional zone.

The final condition is shown in figs. 32 and 33, Pl. 22. The blastoderm is now a compact columnar epithelium and for the first time has become sharply demarcated by its inner cell-walls from the now gradually increasing cytoplasmic reticulum of the yolk. That portion of the periplasm which has not been absorbed into the blastoderm condenses into a structureless limiting-membrane investing the yolk.

These events do not occur at a uniform rate over the whole surface of the blastoderm, but are more advanced at the hinder than at the anterior pole. This is readily seen in any incompletely developed blastoderm; in Text-fig. 3 B, for example, 18 hours after laying, the cells at the anterior end are larger and more sparse than those in the hinder half—contrast in this respect the mature blastoderm shown in Text-fig. 3 D.

For various insects both synchronous and heterochronous division of the blastoderm-cells have been described. In Calandra a marked synchrony of mitoses undoubtedly occurs in the early blastoderm, entrance into the periplasm being probably the co-ordinating stimulus. Later, however, mitoses are seen at random over the whole blastoderm. The existence of co-ordinating factors, even in later blastoderms, is however shown by the occurrence, at times, of considerable areas of cells all in the same stage of mitosis, with the surrounding cells at rest.
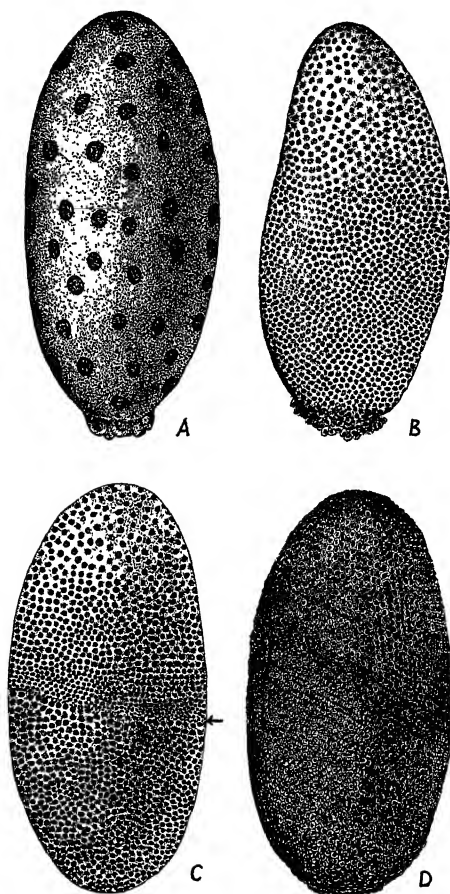
TEXT-FIG. 3.

Blastoderm formation. A, newly formed blastoderm; B, more advanced; C, late blastoderm with wave of mitosis (indicated by arrow) passing over it; D, mature blastoderm, with cell-walls visible even in surface view. Germ-cells prominent in A and B; in C they are already level with the surface, but still protrude a little in D.

The most remarkable form of co-ordination encountered in Calandra is seen in the production of a complete ring of mitoses running transversely round the blastoderm. Only two

examples of this have been encountered, both from late blasto-
derms. One of these has been drawn in Text-fig. 3 c, the ring
of mitoses being indicated by the arrow. Every nucleus in the
ring is in mitosis, and there is not another visible in the whole
blastoderm. Judging by the small size of the nuclei anterior
to the ring, a wave of mitosis is passing backwards along the
blastoderm.

B. The Yolk-cells. These arise mainly by proliferation
of the cells that are left behind in the yolk at blastoderm forma-
tion. In many insects re-entrance of blastoderm-cells into the
yolk has been described, particularly convincingly in Gryllo-
talpa (Heymons) and Campodea (Uzel), where they arise
exclusively in this way. But beyond the extrusion of an occa-
sional degenerated cell from the blastoderm into the yolk, no
evidence for its occurrence in Calandra has been seen. In
very young blastoderms cells are occasionally encountered,
attached to its under surface, and with radial spindles (fig. 29,
Pl. 22). Whether they will later become incorporated into the
blastoderm, or will pass back into the yolk cannot be deter-
mined. As a source of yolk-cell formation they are unimportant.

Proliferation of yolk-cells during the blastoderm period is
very considerable, the 20–30 cells that occur at its inception
increasing up to about 700 in the mature blastoderm; these are
much diminished in size and are not scattered uniformly through
the yolk, but, as described by other authors (Heider, Heymons,
Marshall and Dernehl, Carrière, Nelson, Paterson) often form
clumps which may contain as many as 20 cells (a few small
clumps are seen in figs. 36, 39, Pl. 22).

On the question of the manner of division of yolk-nuclei—
whether by mitosis or amitosis—there is much difference of
opinion. Cholodkowsky (Phyllodromia), Heymons (For-
ficula), and Johannsen (Diachrisia) failed to find any
indication of mitosis, whereas Heider (Hydrophilus), East-
ham (Pieris) and Nelson (Apis) record it, Friedrichs (Dona-
cia) and Marshall and Dernehl (Polistes) describing mitosis
in earlier phases, to become replaced later by amitosis. In
Calandra mitosis of yolk-cells is commonly seen in early
blastoderms (fig. 29, Pl. 22). But in more advanced blastoderms

only occasional preparations show mitosis, but then the majority of cells are in this condition. We have, for instance, one egg with 150 yolk-cells, of which 80, all in the anterior half, are in division ; the remainder, in the hinder half, are all at rest. Synchronization of mitoses, with long intermittent periods of rest, is evidently occurring, mitosis being therefore easily overlooked. Whether amitosis also occurs it is impossible to say.

A feature of these late blastoderms is the large number of degenerate yolk-nuclei which they exhibit. Lecaillon, Friedrichs, and Nelson have already referred to these in other insects. Multipolar mitosis has not been observed. A clumping of chromosomes in dividing nuclei, indicating possible degeneration, as described by Nelson for the honey-bee, is frequently seen.

4. The Germ-cells.

These become recognizable at the time of blastoderm formation. They arise, like the blastoderm-cells, by migration of cleavage-cells into the periplasm. They occur at the hinder pole of the egg, but differ from the blastoderm-cells in that they protrude very prominently beyond the surface (Text-fig. 3 A) ; they are distinguished also by their very rich content of yolk, which they carry with them from the interior of the egg.

As Inkmann has already found for Calandra granaria, a 'Keimbahnplasma' comparable to that described for other insects (v. Hegner, 1914) is not visible.

Proliferation of the germ-cells occurs till there is produced a cluster of still very large cells forming an exceptionally prominent mass at the hinder end of the blastoderm (fig. 30, Pl. 22; Text-fig. 3 B).

At about the end of the first day the germ-cells become withdrawn to the level of the blastoderm surface (Text-fig. 3 c; fig. 33, Pl. 22). By this time they have usually attained that characteristic appearance by which we can readily recognize them in later embryos, the cells being large, with prominent rounded pale nuclei, while the cytoplasm is distinctly eosinophil. Although the yolk-grains are sometimes evident in the germ-cells throughout the embryonic period, they usually soon cease to be conspicuous.

In recent papers—Pierantoni (1927), Buchner (1930), Mansour (1930), Murray and Tiegs (1935)—the peculiar relationship that exists between certain tissues of C a l a n d r a o r y z a e and a bacterial organism has been described. Apart from the specialized bacteria-bearing cells (mycetocytes) that occur in association with the intestine such cells are present also at the tips of the ovarian tubes, whence they infect the eggs, amongst the yolk-grains of which they may be seen. The testis is devoid of mycetocytes.

An association between the bacteria and the germ-cells begins at a very early stage of development. As cleavage progresses the bacteria, hitherto sparsely scattered, begin to accumulate at the hinder pole of the egg, where they become increasingly conspicuous as the germ-cells develop (figs. 30, 32, 33, Pl. 22). In some preparations (haematoxylin staining) they appear merely as an amorphous mass, in others a feltwork of bacteria is visible, while in others again the individual organisms are seen as comparatively large bacteria.

From this mass individual organisms now begin to move out (fig. 30, Pl. 22), and, passing through the secondary periplasm, penetrate into the germ-cells, within which they accumulate in clusters. This occurs irrespective of whether the embryo will become male or female.

The mycetocytes of the ovary develop very early. At the time the germ-cells are becoming withdrawn level with the blastoderm, isolated cells from the latter migrate from the sides into the mass of bacteria (fig. 32, Pl. 22). They are young mycetocytes, and do not appear in the male. Only their nuclei are recognizable, their cytoplasm being entirely obscured by the bacterial content which they acquire.

When later the germ-band develops, the mycetocytes become attached to the mass of germ-cells and thereafter remain in association with them. They are now once more seen as individual cells ; sometimes masses of bacteria are still visible within them (fig. 34, Pl. 22), but usually the bacteria pass out of recognition, the cells being distinguishable from the germ-cells only by their deeper staining with haematoxylin, in contrast to the pale vacuolated eosinophil protoplasm of the latter (fig. 60, Pl. 23).

Within the germ-cells, also, the bacteria are usually visible, but are, of course, much sparser than in the mycetocytes.

In the male, where mycetocytes are absent, the bacteria mostly remain in the yolk.

5. Formation of Germ-band and Embryonic Membranes.

The earliest indication of differentiation of the germ-band from the blastoderm is seen at the end of the first day; the
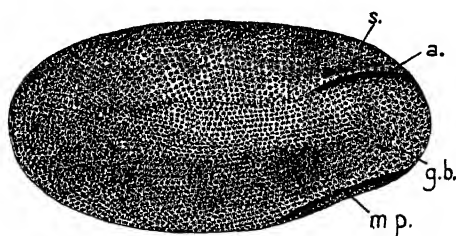
Text-fig. 4.

Differentiation of germ-band from the blastoderm—anterior end to the right. *g.b.*, germ-band; *s.*, serosa; *a.*, developing amniotic fold; *m.p.*, developing median plate.

germ-cells at this period have already become withdrawn to the level of the blastoderm.

Starting in the front half of the egg, and gradually extending backwards, the blastoderm, hitherto of uniform thickness, begins to thin out dorsally owing to its columnar cells becoming gradually flatter. Simultaneously the lateral and ventral walls increase in thickness, the cells becoming irregularly pushed together probably in consequence of the thinning out that occurs along the dorsal surface (fig. 35, Pl. 22). From the thin dorsal part the serosa will form; the thick ventral portion will give rise to the germ-band.

An entire embryo at a slightly later stage of development is shown in Text-fig. 4; a transverse section through the anterior part of the same embryo is depicted in fig. 36, Pl. 22. (Sections towards the hinder end are still similar to that shown in the previous illustration.) In the entire embryo, drawn in lateral

view, the outlines of the germ-band are recognizable, the nuclei showing a tendency to irregular longitudinal alignment, while in the serosa they radiate away from the germ-band. The latter has already grown farther back, being now about three-quarters the length of the egg. The histological structure of the parts is plainly shown in the transverse section; the developing germ-band, it will be noted, has become sharply demarcated from the serosa, while its cells have again formed a regular epithelium.
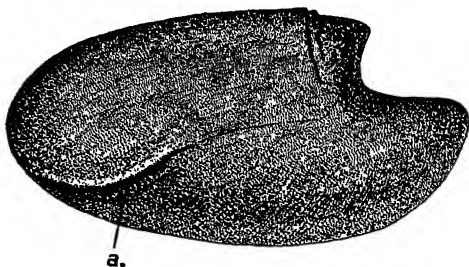
TEXT-FIG. 5.

Development of dorsal flexure. Note deep invagination of germ-band into yolk at posterior pole of egg (to right). *a.*, amniotic fold.

Differentiation of the germ-band has already begun with the formation of a ventral flattening, the median and two lateral plates thereby becoming recognizable (fig. 36, Pl. 22). It begins very early, and proceeds from in front backwards; in the embryo shown in Text-fig. 4 it has already extended back a third the length of the egg.

The period at which amnion formation begins varies. We have one egg in which its formation has preceded the development of the median plate; while in another the median plate has already completely invaginated to form the inner layer before the amnion has begun to appear. In some embryos (e.g. fig. 37, Pl. 22) the development of the amnion on one side is well advanced before that on the other has even begun. As Heider has already observed for Hydrophilus there is a remarkable lack of synchronization in the development of various parts of the embryo at this early period.

Like the germ-band itself the amniotic folds develop from before backwards. In Text-fig. 4 a fold is just beginning to appear as an inconspicuous ridge at the anterior pole of the egg, more advanced stages of its development being shown in Text-figs. 5 and 6. On either side the folds grow downwards along the
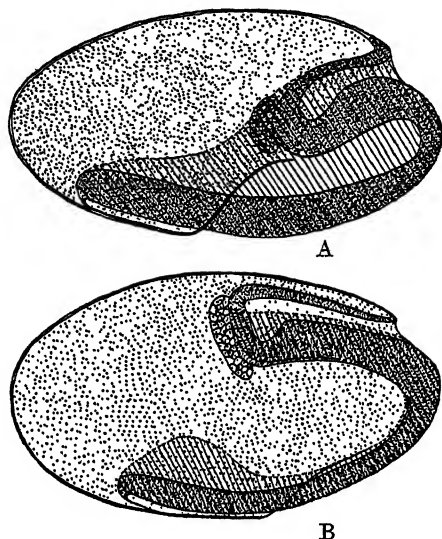
TEXT-FIG. 6.

Two embryos showing development of dorsal flexure and of amniotic cavities; drawn as transparent objects. (Anterior end to left.) serosa—light dotting; germ-band—lines; optical section of germ-band—heavy dotting; note mass of germ-cells at tip of invagination.

junction of the lateral plates and serosa (fig. 37, Pl. 22); it should be noted that the folds are, from the beginning, two-walled, differing in this respect from those of certain Lepidoptera— Pieris (Eastham), Diachrisia (Johannsen). Although mitoses are occasionally seen in the serosa, growth of the embryonic membranes seems to occur chiefly on the thickened zone of insertion of the amnion on to the germ-band.

Fusion of right and left amniotic folds, as they grow downwards under the vitelline membrane, occurs in the mid-line, proceeding as usual from before backwards.

While this portion of the amnion is forming, the germ-band itself is becoming gradually narrower, owing to invagination of the median plate (section 7), till eventually it becomes confined to the lower surface of the egg. The serosa, where it invests the yolk, correspondingly enlarges, while its cells become continually flatter and thinner (cf. figs. 37, 39, and 40, Pl. 22).

At the same time the development of the dorsal flexure of the embryo begins, the method of amnion-formation associated with it being quite different from that seen in the lower half of the egg. The dorsal flexure arises by the germ-band elongating over the hinder pole of the egg on to its dorsal surface. In so doing, however, it does not follow the contour of the egg, but becomes deeply invaginated into the yolk. This is shown in the embryo drawn in Text-fig. 5, while a section through this region, subsequently cut from the same embryo, is drawn in fig. 38, Pl. 22: the invagination is so deep that the embryo at its hinder end is almost crescentic in cross-section; the roof of the invagination is not included in the section for it has been cut rather far to the rear. In later embryos, however, the roof itself extends towards the hinder pole of the egg (Text-fig. 6 A), while the invagination becomes more spherical. The invagination itself, meantime, grows farther along the dorsal surface under the serosa, till eventually at about the twenty-sixth hour it reaches the anterior pole.

The invagination has in the meantime become more dorso-ventrally compressed; it is the amniotic cavity (Text-fig. 6 B; figs. 39, 40, Pl. 22). Its floor, which is very thick, is the dorsal flexure of the germ-band and is much narrower here than along the ventral surface of the egg. The amnion, which forms the roof of the 'dorsal amniotic cavity', is much thicker at this period than the ventral amnion, its cells being large and columnar, and, unlike those of the latter, frequently showing mitoses. The serosa which invests the amnion is, as elsewhere, composed of thin flat cells.

As may be expected the deep invagination of the germ-band, as it makes its way forward under the dorsal serosa, is apt to produce at first considerable surface deformation. (The embryo in Text-fig. 5 shows only a little of this.) But the embryonic

membranes soon readjust themselves under the vitelline membrane and any surface distortion soon disappears.

Closure of the amniotic cavity, by fusion of the dorsal and ventral amniotic folds, occurs at the posterior pole of the egg.

In the embryos of many insects a peculiar 'primary dorsal organ' or 'precephalic organ' (Claypole) has been described, of
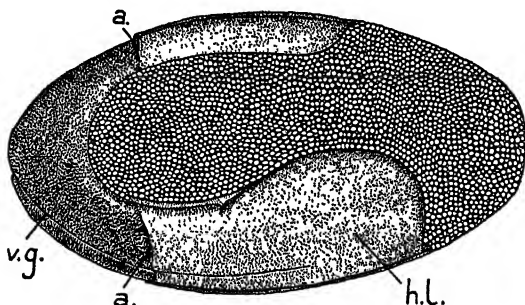
TEXT-FIG. 7.

Embryo at beginning of segmentation; ventral groove present; dorsal and ventral amniotic folds not yet fused. *a.*, hinder margin of amnion; *h.l.*, head-lobe; *v.g.*, ventral (gastral) groove.

uncertain function and homology. It seems to be very common among the apterygote insects (Lemoine, Claypole, Wheeler, Uzel, Philiptschenko), but has been reported also in some of the higher insects—D o n a c i a (Hirschler), A p i s (Nelson), S c i a r a (Du Bois), C o r y n o d e s (Paterson). It appears as a dorsal thickening of the blastoderm at about the time of formation of the germ-band. In C a l a n d r a no trace of it could be found.

6. Segmentation of Germ-band and Development of External Form of Embryo.

A. S e g m e n t a t i o n.—In the foregoing account the development of the germ-band has been given to the stage where it has grown up over the hinder pole of the egg, and extended along its dorsal surface to the anterior end. Thereby it attains its maximum length. In some embryos it actually grows down again over the anterior pole of the egg on to the ventral surface.

While these events are in progress, and before the germ-band has yet attained its maximum length, segmentation begins, the segments appearing in regular succession from before backwards,
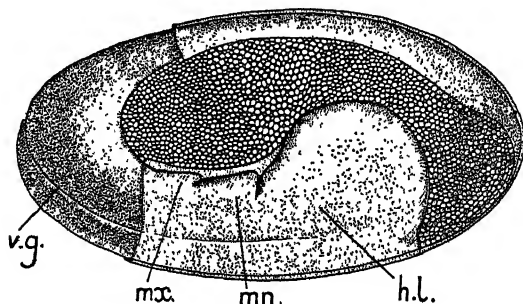
TEXT-FIG. 8.

Early stage of segmentation, with first two gnathal segments defined. Amniotic folds not yet fused. *h.l.*, head-lobe; *mn.*, mandibular segment; *mx.*, maxillary segment; *v.g.*, ventral groove.
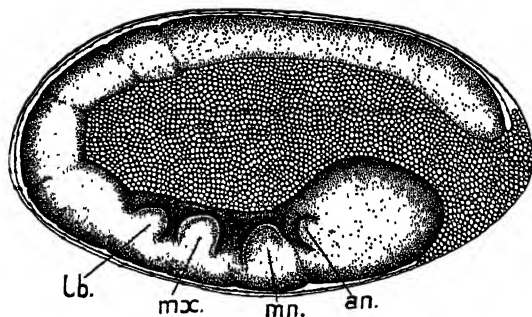
TEXT-FIG. 9.

Embryo with segmentation extending on to beginning of abdomen; antenna and gnathal appendages present, thoracic appendages not yet formed. Amniotic cavity closed (embryonic membranes shown in optical section). *an.*, antenna; *mn.*, mandible; *mx.*, maxilla; *lb.*, labium.

without the occurrence of macro-segments such as have been described by Ayers (1884), Graber (1890), Nusbaum (1889), and Hirschler (1909). The earliest embryo which we have showing indication of segmentation has been drawn in Text-fig. 7; the

head-lobes (protocephalic segment), already evident in Text-fig. 6 B, are clearly defined, and there is an indication of the furrow demarcating it from the mandibular segment.

An embryo at slightly later stage is shown in Text-fig. 8; the head-lobes have become considerably enlarged by spreading
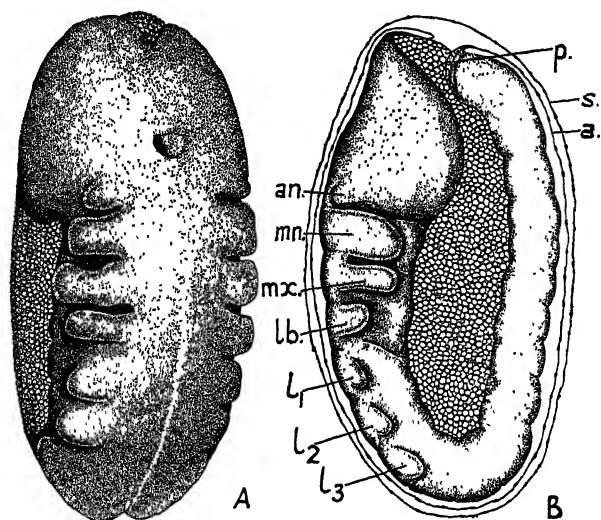
TEXT-FIG. 10.

Embryo with completed segmentation; thoracic appendages developed. A, nearly ventral view; B, the same embryo in lateral view, with embryonic membranes in optical section. The stomodaeum is shown in A, while in B the proctodaeum has been indicated in optical section. *a.*, amnion; *an.*, antenna; *l₁₋₃* legs; *lb.*, labium; *mn.*, mandible; *mx.*, maxilla; *p.*, proctodaeum; *s.*, serosa.

up the sides of the yolk, the outlines of the mandibular segment have become better defined, while a faint indentation marks the future labial segment.

In Text-fig. 9 is shown an embryo with now well-defined gnathal segments, while the segmentation behind has extended to the level of the first abdominal segment.

Segmentation of the abdomen is completed at about the end of the second day of development. An embryo in this condition is shown in Text-fig. 10. In the abdomen eleven segments are

to be counted, the last being the largest. In some insects there is definite evidence for the occurrence of a twelfth segment (telson) bearing the anus. It has been observed by Heymons (1895 *a*, 1897 *a*) in the germ-band of G r y l l o t a l p a and L e p i s m a , while in C a r a u s i u s Wiesmann (1926) found a diminutive twelfth segment, which became absorbed into the
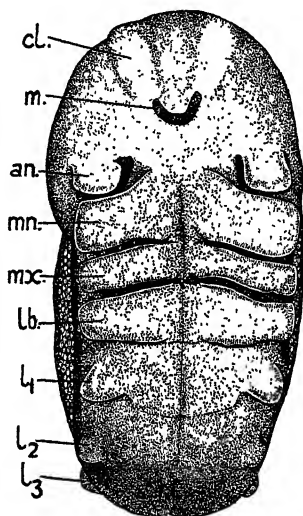
TEXT-FIG. 11.

A rather later embryo than the foregoing, showing paired clypeo-labral 'Anlage' (*cl.*); *m.* mouth. Other lettering as in Text-fig. 10.

proctodaeum in later embryos. A twelfth segment has also been described by Strindberg (1913) for E u t e r m e s and is stated to occur in certain Hymenoptera—C h a l i c o d o m a (Carrière), A p i s (Nelson). In C a l a n d r a when later the proctodaeum develops this arises not on, but behind, the eleventh segment, and this was also observed by Heymons for F o r f i c u l a and P e r i p l a n e t a , while Graber's (1890) illustration of the germ-band of L i n a shows the same. A terminal anus-bearing telson must therefore be regarded as proved for the insect abdomen, even though in many it has become reduced to vanishing point (cf. Heymons, 1895 *b*).

By about the sixtieth hour the process of shortening begins, at the completion of which, early on the fourth day, the definitive larval form is recognizable. The process is initiated by a forward movement of the embryo along the ventral surface of the egg, the head-lobes being thereby carried on to its anterior pole (Text-fig. 12); but beyond this slight movement there is nothing
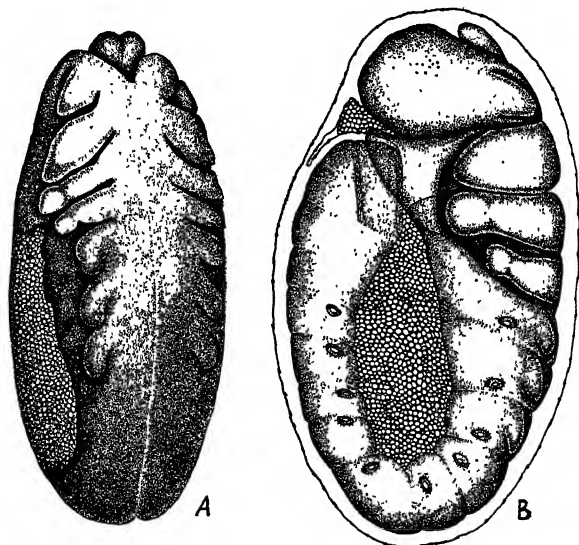
TEXT-FIG. 12.

Embryo in which oral segment has moved on to front pole of egg. A, nearly ventral view; B, lateral view of same embryo. Labrum prominent; legs enlarged; 10 stigmata; proctodaeum tubular (shown in optical section); amnion no longer separable from serosa.

corresponding to the remarkable blastokinesis undergone by the embryos of Orthoptera. The condition of the embryo at about the seventieth hour is shown in Text-fig. 13, while in Text-fig. 14 is shown an embryo early in the fourth day, the shortening being now completed while the posterior end of the embryo has reverted to its position at the hinder pole of the egg. The probable function of these remarkable movements is to bring the embryo in contact with as large a surface of yolk as possible.

Meanwhile the germ-band has gradually widened. The expansion of the head-lobes laterally over the yolk has already been referred to. For the post-cephalic segments this occurs much later, and it is not till the stage of shortening of the
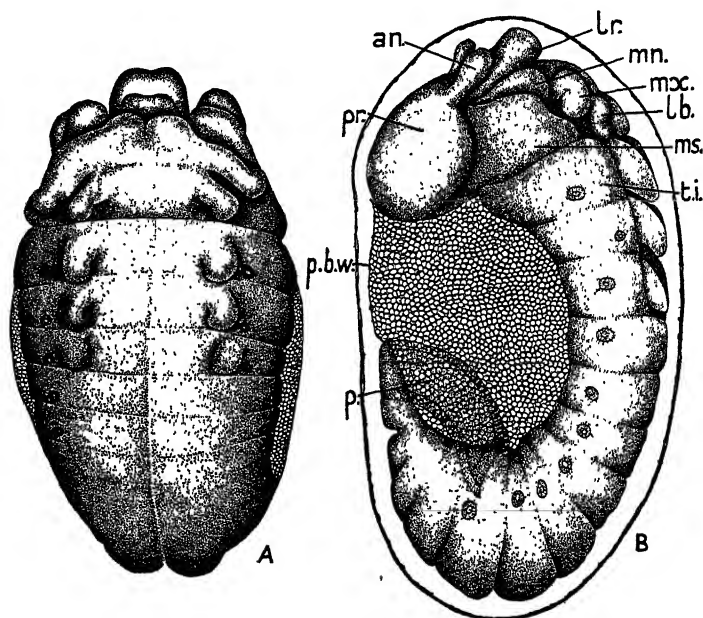
TEXT-FIG. 13.

Embryo undergoing shortening. A, ventral view; B, lateral view of same embryo. The enlarging proctodaeum is shown in optical section. The investing membrane is composed of amnion, serosa, and serosal sheath. *lr.*, labrum; *ms.*, mandibular segment; *p.b.w.*, provisional body-wall (in optical section); *pr.*, protocephalic segment; *t. 1.*, first thoracic segment. Other lettering as in Text-fig. 10.

embryo has set in that it becomes at all active (cf. Text-figs. 10 and 12). By the time the embryo has completed its shortening the yolk has become entirely enclosed at the front and hind poles of the egg, though, in the middle, closure is slower, the egg being at its thickest here.

With the shortening of the germ-band the furrows between

the segments become better defined, and they extend round the embryo as it gradually envelops the yolk.

B. Stomodaeum and Proctodaeum. The stomodaeum is the first to appear. It arises as an oval depression in the middle of the head-segment, usually at the time of formation of the mandibular segment, though in exceptional cases its appearance is delayed till after the formation of the remaining
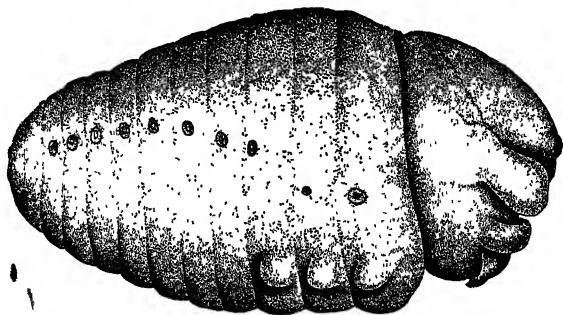
TEXT-FIG. 14.

Embryo on fourth day. Embryonic membranes not drawn. Legs still prominent; suture between protocephalic and mandibular segments still distinguishable.

gnathal segments. Later the depression becomes crescentic (Text-fig. 11). When, during the third day the head moves on to the anterior pole of the egg, the stomodaeum is carried with it, and thereby comes to lie horizontally with its opening forwards instead of downwards as in the earlier embryo.

The proctodaeum arises rather later. It develops as a wide, dorso-ventrally compressed ingrowth into the yolk from the hinder end of the germ-band. It usually lies horizontally (Text-fig. 10 B), though in some exceptional embryos, in which the germ-band does not reach the tip of the egg, it grows vertically downwards. Its relation to the germ-band and amniotic cavity is best shown in sagittal sections. Several stages in its early development are shown in figs. 60, 61, 62, 63, 64, Pl. 23. From the beginning the (true) ventral wall is very thick, the dorsal wall on the contrary thin, and merges into the amnion. A sharp line of demarcation between amniotic and proctodaeal cavities

is, in fact, not recognizable at this period, a fact which has led Inkmann to refer to the latter as 'hinder amniotic cavity'. Actually it is the lumen of the proctodaeum, the structure to which he assigns the latter name being the first pair of malpighian tubes (cf. figs. 60–4, Pl. 23).

The proctodaeum arises then, not within the limits of the eleventh abdominal segment, but behind it. This fact, as already noted, argues for a terminal twelfth segment, that has become reduced to vanishing point.

C. The Head and its Appendages.—This develops out of the head-lobes (protocephalic segment) in which there is no further external indication of segmentation; and from the three succeeding gnathal segments, viz. mandibular, maxillary, and labial.

Of the appendages the mandibular are the first to appear, followed by the maxillary and labial, the antennae not arising till rather later. There are no appendages associated with the intercalary segment. This segment is, in fact, very degenerate in Calandra, and is distinguishable only by its neuromere and by an inconspicuous mass of mesoderm. The development of the labrum is much delayed.

The antennae arise, as usual, as two small backwardly directed papillae from the postero-ventral region of the protocephalic segment, and as in all insects where their formation has been adequately studied, are post-oral in position.

The gnathal appendages arise as prominent, rapidly enlarging laterally projecting outgrowths from the under surface of the corresponding segments (Text-fig. 9; fig. 44, Pl. 22), the mandibular being the largest.

At about the end of the second day the appendages of the maxillary and labial segments become constricted into a large basal and a smaller distal part (Text-fig. 12). The distal lobe will give rise to the palp; from the proximal part will form the cardo and the mala. It should be observed that the bilobed condition arises by transverse constriction, and not, as in some insects (e.g. Donacia, Hirschler, 1909) by the secondary addition of a basal part (cardo and stipes) to the distal palp.

Till now the head-segments lie entirely on the ventral surface

of the egg, extending in some embryos nearly to its hinder pole (Text-fig. 9). But with the movement of the embryo above described the protocephalic segment is now carried forward, and so completely occupies the anterior pole (Text-fig. 12).

The latter segment meantime has considerably enlarged, its line of demarcation from the hinder segments being still sharply defined (Text-fig. 12). Of the gnathal segments the mandibular also becomes enlarged. The maxillary and labial however do not share in this enlargement, but, probably owing to invagination of their sternites into the stomodaeum (see below), decrease markedly in size, and become confined to a region ventral to the mandibular segment. The prothoracic segment has meantime also become enlarged, and as the maxillary and labial move into their definitive position, spreads upwards to impinge on the mandibular segment, and eventually almost reaches the hinder margin of the protocephalic. An early stage of this is shown in Text-fig. 12, a later in Text-fig. 13.

It is about this time that right and left halves of the protocephalic segment begin to fuse in the dorsal mid-line, to be followed later by similar fusion in the mandibular segment. The anterior suture of the mandibular segment with the protocephalic becomes increasingly difficult to identify; its position in the definitive head capsule is approximately indicated in Text-fig. 14, and this corresponds fairly closely with that observed by Heymons (1895 b) in Forficula, where the line of fusion is indicated by a well-defined transverse suture demarcating vertex from frons, and is visible even after emergence. The hinder limit of the mandibular segment is impossible to define; it is quite evident, however, that the latter segment gives rise to the greater part of the hinder wall of the head capsule.

By the beginning of the fourth day the head has become well defined, being now sharply demarcated from the thorax (Text-fig. 14). It has become so enlarged by now, that it is to some extent even invaginated into the prothorax, which has, in the meantime, assumed its adult proportions.

There is much variation in time of appearance of the labrum, for while in some embryos it arises before segmentation of the

germ-band is yet completed, in others (e.g. Text-fig. 10) there is no sign of it, even though all the remaining appendages are formed. It arises by the formation of a pair of ridges—the clypeo-labral 'Anlage'—in front of the stomodaeum (Text-fig. 11). The further development is similar to that described by Hirschler for Donacia. Below, the ridges converge on to the stomodaeum. This portion will become the clypeus, while the labrum itself develops from the more distal paired portion, the process involving a reversal in the position of the two. By about the end of the second day, when the protocephalic segment has moved on to the anterior pole of the egg, the labrum has usually enlarged, and projects as a prominent outgrowth beyond the head (Text-fig. 12). Growth of the labrum now occurs in such a way that these paired outgrowths shift into a position below, i.e. oral to, the clypeus 'Anlage', and so come to overhang the mouth (Text-fig. 13 B). At first still paired (Text-fig. 12 A), they soon fuse to a single process (Text-fig. 15).

Owing to change in shape of the protocephalic segment the antennae, now considerably enlarged, have, in the meantime moved on to the sides of the head, and to their definitive position anterior to the mouth (Text-fig. 13 B).[1]

The position of the mandibles also greatly alters, for they come to lie at the sides of the mouth, undergoing at the same time a rotation in such a way as to direct their free ends towards the mouth (Text-figs. 13, 15).

The maxillae undergo a comparable change, coming to lie postero-lateral to the mouth, with their extremities directed towards it. The formation of the palp by transverse constriction of the appendage has already been referred to. The mala appears to arise as a blunt outgrowth from the basal half. An early stage

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[1] The post-oral position of the antennary segment is usually accepted by morphologists. Holmgren (1909), however, argues for its pre-oral position, on the ground that the deutocerebral commissure is pre-oral, while the labrum is innervated from the tritocerebral ganglion, and should therefore be part of the third segment. It is difficult to evaluate these facts. The position of the mouth of annelids on the first segment cannot be ignored; while the position of the commissures seems to be a secondary consequence of the final position of the ganglion—e.g. in Scolopendra the trito-cerebral commissure is pre-oral, in Scutigera post-oral as in insects.

in the transformation is shown in Text-fig. 15 B—palp and mala are visible, the cardo lying underneath, and therefore not in
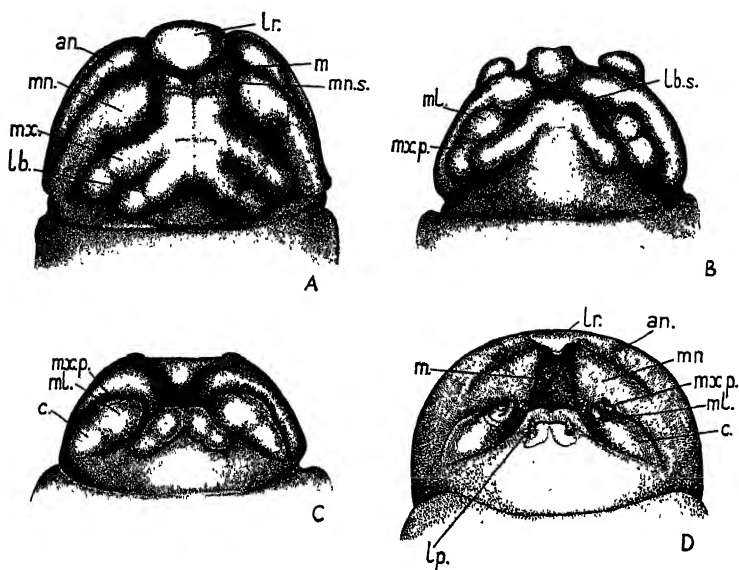
TEXT-FIG. 15.

Development of head; all drawings in ventral view. A, from an embryo rather earlier than that drawn in Text-fig. 13; B, from embryo shown in Text-fig. 14; C, from embryo late on fourth day; D, from embryo shortly before emergence, showing beginning of chitinization (heavy black). *an.*, antenna; *c.*, cardo; *lb.*, labium; *lb.s.*, labial sternite; *l.p.*, labial palp; *lr.*, labrum; *m.*, mouth; *ml.*, mala; *mn.*, mandible; *mn.s.*, mandibular sternite; *mx.*, maxilla; *mx.p.*, maxillary palp.

view. In Text-fig. 15 C the rotation has occurred, all the parts being visible. The structure of the appendage shortly before emergence is shown in Text-fig. 15 D.

The labial appendages meet in the mid-line behind the mouth; the basal parts fuse, the palps becoming directed orally (Text-fig. 15 C, D).

These events are attended by the invagination of the sternites of the gnathal segments into the stomodaeum to form the floor of the mouth, for no part of the head-capsule develops from them.

In Text-fig. 15 A is shown the ventral view of an embryo which has been killed at the time when the mandibular sternites were just undergoing invagination. In Text-fig. 13 A this invagination is complete and the maxillary sternite has advanced to the rim of the mouth. A later stage still is shown in Text-fig. 15 B, the labial sternite being now in course of invagination. When this is eventually completed it allows the labial appendages to approach and unite into the labium (Text-fig. 15 c).

This remarkable dissociation of the sternites from the rest of the segments was first observed by Heymons (1895 b) for Forficula and certain Orthoptera, by Uzel (1898) for Campodea and Tomocerus, and by Holmgren (1909) for Eutermes. In all these cases it gives rise to the hypopharynx. In Calandra, however, where a hypopharynx is absent, the process results merely in formation of the floor of the mouth. In Isotoma according to Philiptschenko (1912) the paraglossae arise in this way.

Chitinization of the head-capsule occurs on the fifth day. A comparison of Text-fig. 15 c and D will show that for maxilla and labium this is attended by considerable shrinkage.

D. Segmentation of the Insect Head.—The difficult task of determining the number of body-segments which, in the extinct ancestors of insects, co-operated in the formation of the complex head-capsule has become a problem mainly for embryology; for the fossil evidence is inadequate, while segmentation in the adult head has become much obscured.

The number of segments involved in the gnathal region may now, with reasonable certainty be taken as three; at any rate, no convincing evidence for the existence of the 'superlingual segment' between the mandibular and maxillary segments is forthcoming. Interest centres, therefore, chiefly in the composition of the large protocephalic segment, i.e. the region anterior to the mandibular segment, the question at stake being whether four or only three segments have entered into its construction.

Commonly it is regarded as composed of three segments, namely an acron (prostomium) fused with (i) the oral segment, devoid of coelomic cavities, with the large protocerebral gang-

lion as its neuromere and labrum as appendage; (ii) the antennary segment, with definite coelom in the embryo, the deutocerebral ganglion being its neuromere, the antenna its appendage; (iii) the premandibular (intercalary) segment, always much reduced in size, with diminutive somite, and with rudimentary appendages surviving in the embryos of primitive forms (cf. Wheeler, 1893; Claypole, 1898; Uzel, 1898; Hoffmann, 1911). It should, however, be observed that the status of the labrum as a true appendage is open to question. Wiesmann's (1926) discovery of mesodermal cavities associated with its 'Anlagen' in Carausius is strong evidence in its favour, as is also its paired origin in many insects. On the other hand, there are species in which it is from the beginning unpaired, a fact which might itself be of no special significance were it not just among the apterygotes that this unpaired origin seems to be general— Lepisma (Heymons, 1897), Anurida (Claypole, 1898), Campodea and Tomocerus (Uzel, 1898), Tomocerus (Hoffmann, 1911), and Isotoma (Philiptschenko, 1912). In Scolopendra also its development as described by Heymons (1901) shows little in common with that of true appendages, for it arises as a median outgrowth from an unpaired clypeus. Its development from paired 'Anlagen' seems therefore to be a secondary acquisition, and, without further evidence, strict comparison with a metameric appendage cannot be unreservedly accepted.

To the three segments above alluded to there must be added, according to Wiesmann's work, a fourth—the reduced preantennary, lying to the side of the mouth. Rudimentary appendage-like structures in this region have already been described by other authors; Wiesmann finds, however, that in Carausius there is a coelomic sac associated with each. This discovery appears then to verify Heymons' (1901) conjecture, based on a study of Scolopendra, that the insect head is constructed out of seven, not six segments, as previously held. It is, at the same time, strange that no reference to such a segment has been made for the embryos of apterygotes.

A study of the neuromeres has, so far, failed to give evidence of more than three segments anterior to the mandibular. On

such data Viallanes (1891) based his pioneering study of head segmentation, and most subsequent work has confirmed his observations. The three ganglia of the protocephalic segment of Calandra are shown in Text-fig. 19; a distinct ganglion corresponding to the pre-antennary segment cannot be distinguished, while the ganglion of the oral segment, on the other hand, is greatly enlarged, its three components being clearly demarcated. There is, however, no certain evidence that these lobes are indicative of distinct segments, or that one of them is the ganglion of the abortive pre-antennary segment, for, according to Heymons, they occur also in Scolopendra, where a distinct pre-antennary neuromere is developed in addition. Wheeler (1893), on such evidence, considered the possibility of an additional segment in Xiphidium, but rejected it. Cholodkowsky (1892), however, accepted it for Phyllodromia. The evidence of Scolopendra is, however, decisively against it. Arguments based on the frontal ganglion need not be considered, as this is part of the visceral system.

The homology of the oral segment is uncertain. Heymons regards it as acron, and homologous with the annelid prostomium, the pre-antennary segment being therefore peristomium. It should be observed that, though the first segment is usually designated 'oral' it is really pre-oral, the mouth being intersegmental, for the pre-antennae lie to the side of it. Goodrich (1897) has advanced cogent reasons to prove that the annelid prostomium is not a separate segment, but in front of the first. If this point be conceded it will affect the status of Heymons' first segment. But, in any case, it seems doubtful whether the homology drawn by Heymons is valid. The first segment of annelids possesses, apart from the archicerebrum (lodged in the prostomium) a ventral ganglion, continuous with the ganglionic chain behind. In Scolopendra an archicerebrum is present, the protocerebral ganglia being therefore presumably the equivalent of the ventral ganglia. It seems more reasonable then to regard the first segment as the equivalent of the first (true) annelid segment. Convincing proof of the homology of the labrum with appendages would decide the matter; but on this point further evidence is needed.

While the evidence is, then, still inconclusive it seems that an acron and possibly seven segments, but at least six segments, have entered into the formation of the insect head.

E. Thorax and Abdomen.—The three thoracic segments develop each a pair of backwardly directed appendages, but none form on the abdomen. The legs appear very early, even before the abdomen has completely segmented. They are much smaller than the gnathal appendages (Text-figs. 10, 11, 12). They do not show any sign of segmentation. They remain prominent till the fourth day, but thereafter gradually retrogress, becoming level with the surrounding body-wall. Here they survive throughout larval life as the imaginal discs from which the legs of the imago will develop.

In the abdomen the number of segments becomes reduced from eleven to ten, due to fusion of the ninth and tenth, on the third day. At the end of the second day the eleventh segment is exceptionally large; but thereafter it becomes reduced in size as the proctodaeum develops at its expense (cf. Text-figs. 13 and 14). It is partially telescoped into the fused ninth and tenth. In the fully grown larva it is very diminutive.

F. Embryonic Membranes.—In their manner of formation, described in section 5, these membranes present nothing unusual. Their later development is, however, peculiar.

Their relationship to the embryo and yolk up to the end of the second day is shown in fig. 59, Pl. 23; the amnion does not exceed the germ-band in width, the yolk being therefore in direct contact at the sides with the serosa. The yolk does not, as in some insects, spread out between amnion and serosa, for it is closely invested by its outer limiting membrane.

Beginning at the hinder pole of the egg and progressing forwards the ventral amniotic cavity now gradually spreads upwards at the sides under the serosa. The amnion becomes thereby stretched into a very delicate membrane, with sparsely scattered nuclei, and adheres usually to the inner surface of the serosa; the inner wall of the enlarging amniotic cavity, scarcely thicker than the amnion, closely invests the yolk. It is a provisional lateral body-wall, and merges below into the thick germ-band (fig. 75, Pl. 24).

On reaching the dorsal surface of the egg the walls of the ventral amniotic cavity now meet and fuse with the dorsal amnion. Dorsal and ventral amnion then merge into one another, and lose connexion with the provisional body-wall, which is itself now merging above into the germ-band. An early stage of this is shown in fig. 83, Pl. 25 (the provisional body-wall is not very extensive here, for the section is through the head-lobes, which cover most of the lateral yolk); a later stage is seen in fig. 75, Pl. 24, and shows the provisional body-wall losing connexion with the amnion. It should be observed that, from their inception, dorsal and ventral amniotic cavities have been in communication at the hinder pole of the egg, and the apparent spreading of the ventral cavity over the sides of the yolk is nothing more than an enlargement of this cavity, which gradually progresses forwards, to form a sac capable of containing the mature embryo, but at a time when the embryo is still in the condition of an elongate germ-band.

At the anterior pole of the egg, between the tip of the proctodaeum and the head-lobes, closure occurs without co-operation of the dorsal amniotic cavity, which is here absent (cf. Text-fig. 10). As Text-fig. 12 B and fig. 65, Pl. 24, show, the provisional body-wall is for a time reflected over the proctodaeal opening, and only later adjusts itself to the surface of the yolk.

The provisional body-wall, then, invests all that portion of the yolk not covered by germ-band; in Text-fig. 13 B, for example, it covers all the part shown as yolk (in the drawing it is indicated only in section at the upper margin). When, during the third day, the germ-band encroaches on the provisional body-wall, it does not displace the latter, but merely incorporates it into itself. Stages in this process are shown in fig. 77, Pl. 24; fig. 115, Pl. 25.

The only noteworthy change in the serosa during these events is the formation of a delicate chitinous sheath on its exterior (cf. figs. 75, 77, Pl. 24; fig. 83, Pl. 25). A similar sheath has been described by Heymons in Lepisma (1897 a). The amnion is exceedingly inconspicuous and usually adheres to the serosa. The embryonic membranes survive thus throughout embryonic life, and must apparently be ruptured by the embryo.

The spreading of the provisional body-wall over the surface of the yolk recalls in some respects Strindberg's (1913) account for Chrysomela; in that insect, however, it becomes displaced by the encroaching germ-band, and degenerates in the yolk as a 'dorsal organ'. Such an organ, indeed, does not form at all in Calandra, which in this respect resembles certain Lepidoptera (cf. Ganin, 1870; Hirschler, 1928; Eastham, 1930 a), where the embryonic membranes survive till emergence of the embryo. In Bombyx according to Ganin the embryo devours the membranes.

7. DIFFERENTIATION OF THE GERM-BAND INTO OUTER AND INNER (LOWER) LAYERS

The early phases of this are described in section 5.

Inner layer formation begins by invagination of the median plate along the greater part of the length of the embryo. Its anterior limit is at the site of formation of the future stomodaeum; while posteriorly it extends as far as the mass of germ-cells, which has become overgrown by the hinder part of the germ-band. The invagination occurs by a bending in of the median plate (fig. 37, Pl. 22), and as this progresses its cells become completely separated off from the lateral plates, which close in beneath it, as the outer layer (figs. 38, 39, Pl. 22). In all embryos examined these events were initiated at the anterior end of the germ-band. The mass of cells thus invaginated is from the beginning a solid cord and is devoid of any trace of a cavity as described for some insects—Hydrophilus (Kowalewsky, 1871; Heider, 1889), Donacia (Hirschler, 1909).

A longitudinal furrow (gastral or ventral groove) marks the line of invagination and of incomplete fusion of the lateral plates; it is seen in Text-figs. 7 and 8, and in figs. 37, 38, Pl. 22. This groove is, however, not long-lived, for the lateral plates soon close in completely under the invaginated mass. At a later period another groove appears in the same place in the outer layer, but this is the neural groove, whose formation is associated with development of the nerve-cord. It is seen in Text-figs. 10–13.

There is much variation in the synchronization of events at this period of development. In some embryos inner layer

formation does not begin till well after the amnion has started to develop; in others again invagination may be complete before there is yet a sign of amnion. In Inkmann's account for Calandra granaria the development is described of a 'ventral groove' preceding amnion formation, which then is said to disappear again, the inner layer arising concurrently with the amnion as a median proliferation of cells, and not as an invagination of a median plate. Unless Calandra granaria is, in this respect, different from Calandra oryzae, Inkmann's 'ventral groove' is merely a case of precocious inner layer formation.

These events occur, and may often be completed, before the dorsal flexure of the embryo has begun to form. The inner layer associated with the dorsal flexure is considerably more massive than that along the ventral surface (figs. 39, 40, Pl. 22), the latter, however, thickening towards the hinder pole of the egg.

Inner layer formation is attended by thickening of the lateral plates. This arises from two causes (i) an elongation of its cells, due chiefly to their developing large vacuoles, (ii) a tendency to form more than one cell-layer.

The cells of the median plate are, like those of the lateral plates, vacuolated and columnar. After invagination they become rounded or polygonal, but retain the vacuoles for a time. Mitoses are now frequently seen among them. As far as could be determined this local cell proliferation alone is the cause of the subsequent enlargement of the inner layer, there being no indication of an acquisition of cells from the lateral plates as described for other species.

The inner layer very early acquires an ill-defined segmentation, consisting of a succession of irregular thickenings, due to local piling up of cells, which correspond in position remarkably with the site of the future body-segments (fig. 41, Pl. 22). In some embryos, however, it is not seen. Precocious segmentation of the inner layer has already been described by Heider for Hydrophilus and by Graber and Eastham for Pieris. In both these species a lateral segmentation is involved, of a kind which does not occur in Calandra. Here the inner layer can readily be examined in its entirety in cleared embryos. It is

much narrower on the dorsal flexure than elsewhere, but only minor indentations appear along its lateral margins, and there is no sign of lateral segmentation.

The cells of the inner layer now begin to spread outwards over the surface of the outer layer, to which they eventually form an almost complete lining. Early stages in this spreading are seen in figs. 40 and 42, Pl. 22; the cells, it will be seen, present no regular form or arrangement. But in the more advanced stage shown in fig. 43, Pl. 22, they have become consolidated into a regular epithelium. Active cell-division accompanies the spreading. Both in the inner and outer layers vacuoles now cease to occur.

The outer layer is, by almost general consent, ectoderm; the interpretation of the inner layer has given rise to much discussion. Heymons, who derives the mid-gut in the pterygote insects from stomodaeum and proctodaeum, regards it as mesoderm, and identifies the endoderm with the yolk-cells. More commonly, however, it is considered as 'mesendoderm', on the ground that the mid-gut also arises from it, either along its whole length, or from cells localized in the 'endoderm rudiments' at its anterior and posterior ends; the yolk-cells are then described as a 'parablast', and not a true germ-layer. The theoretical discussion will be left to a later section (9 c); here we confine ourselves to matters of direct observation.

Now in C a l a n d r a the mid-gut has a bipolar origin, and arises not from anterior and posterior endoderm rudiments (for these do not occur) but, as Mansour (1927) first showed, from the stomodaeum and proctodaeum (section 9 B). The inner layer therefore plays no part in its development. Mansour identifies the endoderm not with the yolk-cells, as does Heymons, but with certain cells that are said to migrate from the inner layer into the yolk and there degenerate. As an important theoretical point is involved, we have examined the matter carefully in numerous serial sections of embryos at appropriate age, but have been unable to find evidence for passage of such cells into the yolk. Mansour writes that the cells 'form a syncytium with deeply staining constituents'. Such cells undoubtedly occur in large numbers in the yolk, even before the inner layer

is formed, but are the clusters of yolk-cells already described (section 3 B), and are readily distinguishable from the cells of the inner layer, for these are, in contrast, rounded, vacuolated, and rather hyaline, and quite different in appearance; nor are transitional stages between the two types of cell found. Apart, therefore, from the impossibility of obtaining evidence for identifying them with endoderm, we must deny the occurrence of such cells in Calandra.

Inkmann (1933) derives the mid-gut from the innermost cells of the inner layer along its whole length, and regards them as endoderm; the histological features by which he distinguishes these cells is, in our material, anything but constant, and in any case it is quite evident that he has altogether misinterpreted the process of mid-gut formation in Calandra.

There is then no adequate reason for identifying any part of the inner layer of Calandra with endoderm.

8. Differentiation of the Inner Layer, up to the Formation of the Coelomic Sacs

A. Segmentation.—During the period of lateral spreading of its cells, the early segmentation of the inner layer, described in the last section, becomes temporarily obscured, and does not reappear till the cells have become ordered into a regular epithelium. This occurs near the middle of the second day, the embryo being at about the stage of development shown in Text-fig. 9. It appears first in the thorax and the labial segment, and spreads backwards along the abdomen, slightly preceding the external segmentation.

This segmentation results in the formation of two lateral rows of somites, separated by a thin median unsegmented strip of mesoderm (figs. 43, Pl. 22; fig. 59, Pl. 23). In the latter the cells form a simple epithelium, one cell thick. In the somites two layers occur—the more internal (splanchnic) layer comprising at first rather flattened cells which multiply and later become cubical; while adjacent to the ectoderm the somatic layer is formed of cells which become gradually more elongate (fig. 45, Pl. 23). At the intersegments the lateral zones are reduced to one cell in thickness (fig. 59, Pl. 23).

In rather later embryos a complete separation of successive somites may be effected (fig. 45, Pl. 23). For Orthoptera this condition is well known; for Coleoptera it seems to be unusual.

The somites lie towards the outer margin of the body-wall, dorso-laterally to the appendages when these are present (figs. 46, 47, Pl. 23).

In the abdomen segmentation extends to the hinder end of the ninth segment. A somite is present in the tenth (fig. 61, Pl. 23), but though demarcated from the ninth is confluent behind with a prominent and still enlarging unpaired mass of mesodermal cells situated in the last segment, behind the germ-cells and occupying much of the space between the proctodaeum and the body-wall (figs. 61, 62, Pl. 23).

In the maxillary and mandibular segments somites do not occur, the lateral zones of mesoderm being not even epithelial in character. In the mandibular segment this mesoderm is much reduced (fig. 44, Pl. 22). In the region of the intercalary segment it is impossible to distinguish a somite; later, however, there is an indication of a vestigial coelomic sac (v. section 14).

Owing to the greater width of the germ-band in the gnathal region the layer of mesodermal cells is apt to be rather thinned out there, this being accentuated by the mesoderm extending as a loose clump of cells into the cavities of the appendages; the antennae, which are post-oral in position at this period, also acquire mesoderm in this way (fig. 59, Pl. 23).

With the subsequent enlargement of the gnathal appendages, particularly the mandibular, these loose clumps of cells become converted into simple epithelial linings for the appendages (fig. 44, Pl. 22). Closed sacs do not form.

At this period, then, the mesoderm of the gnathal segments consists of a thin sheet of cells, slightly thickened laterally, but much reduced in the mandibular segment, with pocket-like extensions into the appendages (particularly mandible), and with a pair of somites in the labial segment.

B. The Pre-oral Mesoderm.—This has, as in all higher insects, a post-oral origin. While the cells of the inner layer spread out over the ectoderm, a small clump of cells remains heaped up immediately behind the developing stomodaeum

(figs. 52, 53, Pl. 23). This must not be taken for 'anterior endoderm rudiment', for it plays no part in mid-gut formation.

From this clump cells now spread forwards round the stomodaeum, and while undergoing active division extend in the form of two sheets laterally along the floor of the protocephalic segment. These two strands of mesoderm become united by a transverse band of cells in front of the stomodaeum, which in this way becomes encircled with mesoderm.

During the latter half of the second day the cavities of the paired labral 'Anlage' have become invaded by cells that migrate from the mesoderm in front of the stomodaeum.

By the end of the second day the pre-oral mesoderm has developed into a consolidated layer of cells on the floor of the protocephalic segment, spreading at the same time, as a layer of scattered cells, up the sides. It remains connected with the post-oral mesoderm by a pair of thick bands to the side of the stomodaeum, representing the premandibular (intercalary) mesoderm. The mesoderm associated with the antenna, which has begun now to move forwards, is very conspicuous.

C. The Coelomic Sacs.—During the second day the somites have enlarged, cell-division being frequently encountered. The coelomic sacs arise towards the end of the second day, their formation being initiated by the appearance of a narrow cleft between the splanchnic and somatic layers.

Probably owing to secretion of fluid into their interior these clefts now expand into prominent cavities. They develop in the somites of the labial to ninth abdominal segments. In the tenth abdominal somite a cavity does not appear, nor does it form in the mesoderm of the mandibular and maxillary segments. In the thorax and the labial segment the coelomic sacs are elongate and oval; in the abdomen when fully developed they are rather more spherical.

The separation between successive somites above alluded to is now no longer seen. Early on the third day, at a time when the germ-band is beginning to shorten, the cavities of successive somites communicate with one another by narrow though well-defined channels in the intersegmental constrictions (fig. 48, Pl. 23), so that the coelomic sacs now form a pair of moniliform

tubes running from the labial to the ninth abdominal segment. While this condition is well known for Orthoptera and Dermaptera, there is some doubt as to its occurrence in Coleoptera. Hirschler (1909) was unable to detect it in Donacia; it has, however, been described by Graber (1890) for Melolontha and Lina, and by Heider (1889) for Hydrophilus. With the use of celloidin embedding, as in the present work, the tendency to form artificial cavities within the tissues is reduced to a minimum.

In the protocephalic segment only one pair of coelomic sacs forms, namely in association with the antennae. They appear later than do the others; not indeed till after the germ-band has begun to shorten, i.e. early on the third day. Although the mesoderm of the antenna has not hitherto shown the characters of a true somite, the coelomic sac which forms within it does not, except for its later appearance and rather small size, present anything unusual. It does not occupy the cavity of the antenna, but lies at its base (fig. 86, Pl. 25), in the main a little to the rear of it (fig. 87, Pl. 25). A premandibular coelomic sac does not form (see further, section 14). In the labrum, also, coelomic sacs do not appear, there being indeed no evidence for the formation even of somites in association with it (cf. fig. 92, Pl. 25); this is noteworthy because Wiesmann (1926) has described definite coelomic sacs in the labrum of Carausius.

The coelomic sacs of Calandra closely resemble those observed for other members of the higher orders of insects, there being nothing comparable to the large, often trilobed cavities, with extensions into the appendages, that are described for the more generalized forms—Orthoptera (Cholodkowsky, 1889; Graber, 1890; Heymons, 1895; Wiesmann, 1926), Forficula (Heymons, 1895), Lepisma (Heymons, 1897 a), Eutermes (Strindberg, 1913); as well as in Scolopendra (Heymons, 1901).

The occurrence of coelomic sacs in Calandra from the labial to the ninth abdominal segment agrees well with Hirschler's description (1909) for Donacia. For the head segments, however, an important difference is to be noted. According to Hirschler there is a well-developed intercalary coelom, from which the cephalic aorta arises, while an antennary coelom

is absent. This is surprising, in view of the fact that an antennary coelom is almost universal in insects (it seems to be absent in Hydrophilus and Pieris), while an intercalary coelom occurs, even as a vestige, in only the most primitive forms. Since Hirschler derives the sub-oesophageal body (q.v.) from the mid-gut epithelium, and not, as usual, from the intercalary mesoderm, it seems very probable that he has misinterpreted this region of the embryo. It is desirable that Donacia should be reinvestigated on this point, as it forms the type for current text-book description.

9. ALIMENTARY CANAL: EARLY DEVELOPMENT.

A. Early Development of Stomodaeum and Proctodaeum.—This has already been alluded to in the general description of the germ-band (section 6 *B*). In the present section their early development only is described; for the later phases see section 11.

(i) Stomodaeum.—In very early germ-bands, at a time when the dorsal flexure has scarcely attained its maximum length, the ectoderm presents a pronounced thickening just anterior to the tip of the invaginated inner layer, a thickening which appears accentuated by the fact that the ectoderm immediately behind it is unusually thin (fig. 49, Pl. 23).

At a rather later period, when the gnathal segments have already appeared, this thickened area is seen in process of invagination; it is the 'Anlage' of the stomodaeum (fig. 50, Pl. 23). Becoming later hemispherical (fig. 51, Pl. 23) it then gradually elongates and becomes more and more cylindrical (fig. 52, Pl. 23).

Even before invagination of the stomodaeum has begun, a slight heaping up of mesoderm cells at the tip of the inner layer is seen. This has already been alluded to in the foregoing section, and must not be mistaken for 'anterior endoderm rudiment'. During the subsequent migration of its cells round the stomodaeum some of them spread on to its postero-lateral wall; but except for this, the stomodaeum remains mainly in direct contact with the yolk.

(ii) Proctodaeum.—In section 6 *B* the development of the

proctodaeum has been described to the stage at which, in the latter half of the second day, it occurs as a horizontal cleft-like ingrowth into the yolk, its (true) ventral wall being thick, but its dorsal wall at this period scarcely thicker than the amnion itself with which it is continuous. Its appearance in sagittal section is shown in figs. 60, 61, Pl. 23; while fig. 62, Pl. 23, represents a transverse section.

Towards the end of the second day the dorsal wall becomes much altered; its cells multiply considerably, and become long and cylindrical, and the wall therefore much thickened (figs. 63, 64, Pl. 23). It grows also considerably in length and extends forwards almost to the level of the eighth abdominal segment.

The relation of the proctodaeum to adjacent parts at about the end of the second day is shown in fig. 63, 64, Pl. 23. Within the eleventh segment the inner layer is very thick and forms a large unpaired mass occupying all the space between the proctodaeum and the body-wall, and separated from the yolk by the still unpaired mass of germ-cells. The first pair of malpighian tubes is beginning to form. The cavity of the proctodaeum has not yet opened on to the yolk.

Till now the proctodaeum is still dorso-ventrally compressed. But a change now occurs whereby it becomes converted into a cylindrical tube, of which the lumen is now sharply distinguishable from amniotic cavity (figs. 65, 66, Pl. 23; fig. 83, Pl. 25). At its most internal extremity, however, where it abuts on the yolk, its roof remains thin, and its cavity dorso-ventrally flattened (fig. 67 B, Pl. 24).

B. The Mid-gut.—The development of this organ still remains one of the most controversial problems of descriptive embryology. The literature on the subject is treated at length by Hirschler (1928) in Schröder's 'Handbuch' and in the special review by Eastham (1930 b). For present purposes the following short summary will suffice:

(i) The mid-gut is formed from the yolk-cells. Although held by many of the older writers, this view now finds support only in Heymons' work on Lepisma (1897 a) and Campodea (1897 b).

(ii) The mid-gut is a derivative of the proctodaeum and

stomodaeum, and is therefore ectodermal. This view originated, it seems, with Ganin (1874) and was upheld by Graber (1890, 1891) for Orthoptera, and Korotneff (1894) for Lepidoptera. With the appearance of Heymons' great work on the Orthoptera and Dermaptera in 1895 the subject first gained prominence, and has since found support in the work of Schwartze (1899) and Johannsen (1929) on Lepidoptera, and of Lecaillon (1898), Deegener (1900), Friedrichs (1906) and Mansour (1927) on Coleoptera.[1]

(iii) The mid-gut cells arise either by delamination, or invagination, or inward proliferation from the outer layer, in complete independence of stomodaeum and proctodaeum, and by a process which is usually regarded as, at least in principle, comparable with gastrulation. In one form or another most authors support this view.

In Eutermes and several Hymenoptera and Coleoptera such endodermal cells are, according to Strindberg (1913) spread throughout the length of the inner layer; and a similar conclusion is reached by Hammerschmidt (1910) and Leuzinger and Wiesmann (1926) for Carausius, by Inkmann (1933) for Calandra and by Paterson (1935) for Corynodes. Anterior and posterior endoderm rudiments are said, then, not to occur.

For most species, however, the bipolar origin, first described by Kowalewsky (1886) for muscids is observed, though some authors admit the participation of a narrow median connecting strand ('Mittelstrang')—Nusbaum and Fulinsky (1906, 1909), Hirschler (1909, 1912), Philiptschenko (1912).

In regard to the origin of the bipolar rudiments, these are said to arise either (i) as thickenings of the inner layer—Kowalewsky (1886) for muscids, Heider (1889) for Hydrophilus, Wheeler (1889) for Blatta and Doryphora, Strindberg (1914) for Vespa, or (ii) as an independent inward proliferation of cells from the outer layer—Carrière and Bürger (1897) for Chalicodoma, Noack (1901) for Calliphora, Nusbaum and Fulinsky (1906, 1909) for Phyllodromia and Gryllotalpa, Nelson (1915) for Apis, ?Strindberg (1916)

[1] Roonwal (Phil. Trans. Roy Soc. B. 227. 1937) upholds this for Locusta. This paper appeared too late for reference in the text.

for Sialis, Eastham (1929) and Henson (1932) for Pieris, Mellanby (1935) for Rhodnius, and Thomas (1936) for Carausius.

It would be an error to regard these diverse views as mutually exclusive, for they relate to insects of the most varied kinds. But in some cases, where identical or closely related species are concerned, comparison is possible. Thus Hirschler's interpretation of mid-gut formation in Donacia differs radically from that of Friedrichs on the same species; while in Carausius the recent account by Thomas is impossible to reconcile with that of Hammerschmidt, and of Leuzinger and Wiesmann.[1] In regard to Calandra Tichomirow derives the mid-gut from yolk-cells, Mansour from stomodaeum and proctodaeum, while Inkmann describes its origin from endoderm cells distributed throughout the length of the inner layer. Our own observations agree essentially with those of Mansour.

(a) Anterior (stomodaeal) Component of Mid-gut.—If an embryo from early in the third day be examined, i.e. at about the stage shown in Text-fig. 12, the mid-gut 'Anlage' is clearly recognizable as far back as the level of the labial segment, where it is seen as a pair of narrow lateral bands lying on the coelomic sacs next to the yolk (fig. 47, Pl. 23). Anteriorly to this the bands widen, forming, just behind the stomodaeum, a complete sheet of cells under the yolk. The cells are usually rather large, pale, vacuolated, and well merit the name 'succulent' that is often applied to them. In rather younger embryos the hinder limit of the mid-gut 'Anlage' may be seen extending to various levels between the stomodaeum and the labial segment. In some embryos at about this period it is represented only by a transverse bar behind the stomodaeum (fig. 59, Pl. 23). Since there is no evidence for a local differentiation of the cells of the mid-gut 'Anlage' from the coelomic sacs, it is evident that there must be occurring a backward migration from the region of the stomodaeum, and it remains to determine the exact origin of its cells. The difficulty

[1] The structure which Thomas regards as posterior endoderm rudiment seems to be the genital rudiment (cf. fig. 13 of Thomas with fig. 6 (p. 142) of Wiesmann).

which has been experienced in elucidating this point reflects the sharply contrasted opinions concerning it which are expressed in the literature. The following account is based on an examination of numerous carefully prepared celloidin sections, in which the fixation and staining left little to be desired; the accompanying illustrations have been drawn, cell for cell, with scrupulous accuracy, and with use of the camera lucida.

The condition of the stomodaeum, immediately before the mid-gut 'Anlage' appears, is shown in fig. 51, Pl. 23 (sagittal section). It is now a hemispherical organ, with pronounced lumen; there is a slight heaping up of inner layer cells just behind it, while others have migrated round, and now appear in front of it. Incidentally, it may be noted, Paracytoid formation (v. section 21) is very active.

Till now the hinder wall of the stomodaeum has preserved its epithelial character. Mid-gut formation is initiated by some of these cells now elongating and extending outwards towards the yolk. A very early stage of this is shown in fig. 52, Pl. 23; the stomodaeum has become cylindrical, the epithelial character of its hinder wall disorganized, and some of the cells have grown long and narrow, but are hardly yet protruding beyond its surface.

A later stage is shown in fig. 53, Pl. 23; the hinder wall of the stomodaeum has now completely lost its epithelial character; it has grown markedly in thickness, and its cells are evidently growing backwards over the heap of inner layer cells.

In fig. 55, Pl. 23, a rather later stage still is shown. The mid-gut 'Anlage' now appears as a compact mass of cells, still connected in front with the hinder wall of the stomodaeum, whose regular epithelial structure is here disorganized. The cells have already begun to assume the peculiar texture of mid-gut cells. The 'Anlage' itself is sharply demarcated from the underlying inner-layer cells. It is important to observe that there is no evidence for regarding it as a differentiation of the invaginated inner layer; nor does it arise, as in Chalicodoma (Carrière and Bürger, 1897) by proliferation of the outer layer, which then invaginates with the stomodaeum, but from the stomodaeum itself. That the inner layer plays no part in its formation is well shown in those embryos where it arises from a more

restricted part of the stomodaeal wall; fig. 54, Pl. 23, will render detailed description unnecessary.

From the mid-gut 'Anlage' which thus arises from the hinder wall of the stomodaeum, cells now spread forwards to the sides of the stomodaeum, over the mesoderm cells, and become the pre-oral mid-gut 'Anlage'. This is well seen in fig. 57, Pl. 23. The drawing is from an embryo cut in horizontal section. The stomodaeum is cut transversely; its hinder wall has lost its epithelial character and the mid-gut cells are seen spreading laterally and then forwards round the stomodaeum. The pre-oral mid-gut cells are to be seen also in fig. 64, Pl. 23. From them forms that part of the mid-gut wall that lies just dorsally to the oesophagus.

In embryos in which the coelomic sacs have begun to open, the mid-gut 'Anlage', though still forming a prominent mass of cells just behind the stomodaeum, has now lost direct connexion with the latter, the posterior wall of which has regained its regular epithelial character (fig. 56, Pl. 23). Backward migration of the mid-gut cells, which has already been described, now begins. It is attended by much cell-division. The post-oral mass thereafter gradually dwindles.

(b) Posterior (proctodaeal) Component of Mid-gut.—This develops much later than the stomodaeal component, for it does not arise till after the germ-band has begun to shorten, i.e. early on the third day, and at a time when the stomodaeal component has already grown back as far as the first thoracic segment.

The blind end of the proctodaeum widens out and begins to bend down over the germ-cells. Simultaneously the dorsal (thin) wall of the proctodaeum opens, thereby giving the yolk direct access to the lumen of the proctodaeum. This early opening of the proctodaeum is noteworthy, since later it again becomes completely closed. Though having direct access to the proctodaeum, the yolk does not, as a rule, enter it, being held back by its limiting membrane.

From the thick ventral wall of the proctodaeum, as it bends over the mass of germ-cells, there grow out two lateral strands of cells which, insinuating themselves between the yolk and the

germ-cells, eventually extend along the mesoderm to the eighth abdominal segment. The median zone of the proctodaeum grows more slowly, but soon it, too, grows down on to the mesoderm, the germ-cells being thereby completely cut off from contact with the yolk. In this way is formed the posterior 'Anlage' of the mid-gut.

As a possible origin of mid-gut from proctodaeum has been the subject of so much discussion it is desirable to illustrate the evidence for it as completely as possible. In fig. 67 A–H, Pl. 24, is shown a series of successive transverse sections of the proctodaeal region of an embryo in which shortening of the germ-band is just beginning (cf. Text-fig. 12). The tubular proctodaeum (cf. fig. 83, Pl. 25) is shown widening out in fig. 67 A, Pl. 24; the roof has become thin, the first pair of malpighian tubes is seen in transverse section, and the base of the second pair is just distinguishable. As the tip of the proctodaeum is approached in the succeeding sections, it is seen gradually widening out. In C and D disappearance of the thin roof is observed, giving thereby direct access of the yolk to the lumen of the proctodaeum. In the succeeding sections the median portion of the ventral wall gradually thins out and disappears, while thickened strands from its lateral margins extend as mid-gut 'Anlagen' as far as the eighth abdominal coelomic sac in figs. 67 G and H, Pl. 24. (Note ninth coelomic sac in C and D.)

The embryo here selected shows the hinder mid-gut 'Anlage' at the very beginning of its formation. The ensuing events may be pictured by reference to fig. 68, Pl. 24. This is from a rather later embryo, the section passing through the eighth abdominal coelomic sac (i.e. at about the same level as fig. 67 G, Pl. 24). The mid-gut 'Anlage' is now a complete sheet of cells shutting off the germ-cells from the yolk.

The divergence of the mid-gut 'Anlagen' from the tip of the proctodaeum, as here described, is best studied in transverse section. Sagittal sections are, however, very instructive. Fig. 64, Pl. 23, shows such a section from an embryo in which the proctodaeum is at a rather earlier stage of development than that shown in the serial section, for it is still a dorso-ventrally compressed cleft. It should be compared with the stage shown in

fig. 63, Pl. 23, where there is yet no sign of mid-gut formation. From the tip of the proctodaeum a few cells are seen spreading over the germ-cells, reaching the level of the eighth abdominal coelomic sac. At the same time the opening of the proctodaeum on to the yolk, which is later so conspicuous (fig. 65, Pl. 23), is just becoming apparent.

Taken by itself this section will not give an adequate picture of these early stages of mid-gut formation; but in conjunction with the transverse sections depicted a clear idea of events will be obtained.

Anterior and posterior mid-gut 'Anlagen' meet, towards the end of the third day, at about the level of the first or second abdominal segment. Although each extends through about seven segments the anterior is much the larger, owing to the greater size of the anterior segments.

C. Discussion.—Before discussing the. bearing of these observations on the germ-layer theory it is desirable to comment on their objective validity.

(*a*) Validity of the Observations.—The difficulty which has confronted most observers on this point is to decide whether the mid-gut cells arise from stomodaeum and proctodaeum, or from the immediately adjacent inner-layer cells.

In the case of the proctodaeal component this difficulty does not arise in Calandra, for the tip of the proctodaeum is, from the beginning, separated from the inner-layer cells by the large mass of germ-cells; as fig. 61, Pl. 23, will show, the large mass of inner-layer cells in the eleventh segment cannot possibly participate in mid-gut formation.

For the stomodaeum the matter is not so simple, there being a close association between it and the anterior clump of inner-layer cells. Is the latter an 'anterior endodermal rudiment', or is it purely mesoderm? MacBride (1914) commenting on the point writes that it is a case 'where the endoderm rudiment becomes distinguishable at a very late stage of development, and where its first origin is impossible to determine with accuracy'. This difficulty was encountered during the present work, and preliminary observations, based on the study of transverse sections, appeared to confirm the orthodox view.

The difficulty with the transverse sections is that the disorganization of the hinder wall of the stomodaeum cannot be detected by their means, the cells which migrate backwards over the piled-up mesoderm having the appearance of originating from the latter. If, for example, the embryo drawn in fig. 53, Pl. 23, had been transversely cut, there would have been no clue to their origin from the stomodaeum, and they would have been interpreted as a differentiation from the inner-layer cells. Moreover, in transverse sections the mid-gut cells usually appear sharply demarcated from the stomodaeum, particularly from its lateral walls (fig. 58, Pl. 23); but this is because they have migrated there from the hinder wall. Reference to fig. 57, Pl. 23, will make this clear.

In sagittal sections, however, the origin of the mid-gut rudiment as an outgrowth from the hinder wall of the stomodaeum itself becomes apparent. The temporary derangement of its epithelial wall, and the backward migration of cells from this part over the heaped-up mesoderm is then obvious, and there is no evidence for a local differentiation from the latter.

To make the demonstration of this very controversial point as objective as possible, three photographs are here offered (Text-fig. 16 A, B, C), representing the same objects as have been drawn in figs. 50, 52, and 55, Pl. 23. In C the mid-gut 'Anlage' is very conspicuous; it is directly continuous with the hinder wall of the stomodaeum, and is sharply demarcated from the inner-layer cells, over which it is growing. Has it arisen from the outer layer and then become drawn in with the stomodaeum as the latter invaginated? Fig. A shows that this is not the case, for there is no indication of it yet, although the stomodaeum has itself become defined. Even in B, with the stomodaeum already cylindrical, it is only just beginning to form, its site of origin being the tip of the stomodaeum.

No claim is made for the generality of this observation, for the opposite conclusion of Nusbaum and Fulinsky (1906) on Phyllodromia germanica, and of Henson (1932) on Pieris brassicae seems equally conclusive for those species. The former work is of special interest because it employs one of the species on which Heymons based his novel views. Nus-

baum and Fulinsky, and Henson both describe a proliferating zone of ectoderm immediately behind the place, or, in Pieris, at the place, where the stomodaeum will later form. From this zone of proliferation both mid-gut rudiment and some meso-
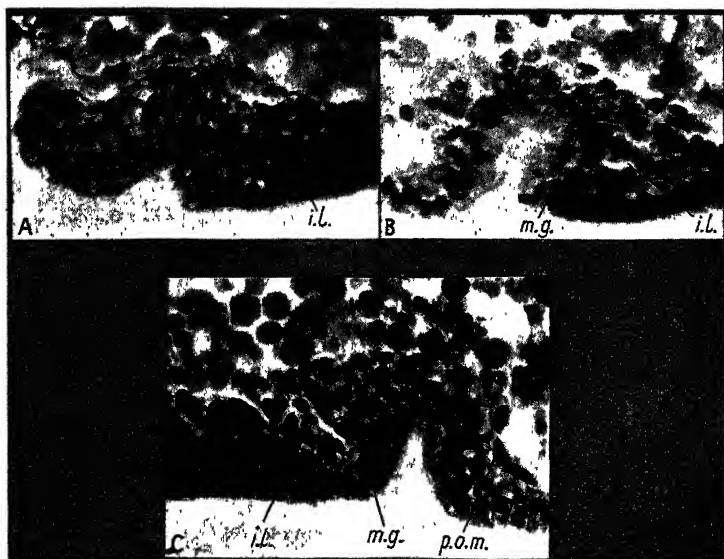
TEXT-FIG. 16.

Sagittal sections through stomodaeal region of early germ-band, with mid-gut in course of formation. For explanation see text. *i.l.*, inner layer; *m.g.*, mid-gut 'Anlage'; *p.o.m.*, pre-oral mesoderm. A few paracytoids appear in B. Photographs by Dr. E. S. J. King.

dermal cells arise. In Phyllodromia this zone of proliferation may become invaginated with the stomodaeum, and so give the false impression of actually arising from the latter. In this way the orthodox view of the mid-gut arising from a kind of blastopore, i.e. common meeting-ground of ectoderm, mesoderm, and endoderm, may perhaps be upheld, while the need of deriving it from the stomodaeum, i.e. conventional ectoderm, is avoided. But for Calandra this explanation will not hold; such a zone of proliferation does not occur here and the mid-gut forms from the stomodaeum itself.

(b) Application to the Theory of Gastrulation, and the Germ-layer Theory.—It is evident that the foregoing observations on the origin of the mid-gut of Calandra involve the validity of the theory of gastrulation and of homology of germ-layers. Heymons has already discussed the implication of such observations in his work on the Dermaptera and Orthoptera (1895) and in his later monograph on Scolopendra (1901); but although the validity of his discussion is open to question it is the observations themselves and not their consequences alone which have been often discredited. Having encountered in Calandra an insect whose development conforms with that described by Heymons, the need arises for again considering the meaning of such observations, particularly as much of the past discussion on the subject is marred by the fact that the principles involved have acquired a variety of meanings with different authors.

In his celebrated 'Studien zur Gastraea Theorie' Haeckel, to whom the term is due, defines the gastrula as a 'monaxial unsegmented hollow body, without appendages, whose simple cavity (Urdarm) opens by an orifice (Urmund) at one pole of the axis, and whose body-wall is composed of two layers of cells—endoderm or gastral layer, and ectoderm or dermal layer'. Its importance lies in the fact that it occurs 'in animals of the most diverse classes, from sponges to vertebrates in the same characteristic form'; and in the special significance which Haeckel attached to it as recapitulating in the ontogeny of the individual the extinct gastraea ancestor of the metazoa. Four varieties of gastrula were described, the insect gastrula belonging to the type designated 'perigastrula'. An illustration, supposedly based on Kowalewsky's (1871) account for Hydrophilus, accompanies the description and shows the invaginating ventral groove designated endoderm, with its cavity the 'Urdarmhöhle'. But to conform to the scheme this gastrula has been constructed in disregard of Kowalewsky's observation that the invaginated layer is not endoderm, nor its cavity an archenteron.

Balfour (1880) showed the error of this interpretation and denied the existence of a gastrula in the ontogeny of insects. The results of subsequent investigation have vindicated his

view, for the ventral groove is a secondary acquisition of higher insects, being absent in Scolopendra and in all the apterygote insects hitherto examined (Campodea, Tomocerus, Lepisma, Anurida, Isotoma) and even in Eutermes and some Orthoptera. Heymons' work on the Orthoptera seems, in fact, to show the groove in process of evolution.

In 1886 Kowalewsky reinstated the insect gastrula, but in a modified form, and this has since been widely accepted. The ventral groove he regards as an elongate blastopore, the endoderm having become confined to its ends, with a long strip of mesoderm intervening. Sagitta is rather unconvincingly cited as analogy. Kowalewsky's scheme derives support from the later work of Nusbaum and Fulinsky, Hirschler, Philiptschenko, and others, who describe in certain species the narrow median band of endoderm (Mittelstrang) connecting the anterior and posterior endoderm rudiments. Whether such a 'gastrula' conforms with Haeckel's definition may perhaps be questioned; but the conception of endoderm arising by invagination at a blastopore has at least survived.

Even the extreme case described by Henson (1932) for Pieris might be reconciled with the theory. Here the mid-gut 'Anlage' arises, together with a small part of the mesoderm, from proliferation of the outer layer, but independently of the inner layer, the sites of proliferation occupying the place where stomodaeum and proctodaeum subsequently invaginate; since 'ectoderm, mesoderm, and endoderm run indistinguishably into one another' here, these regions may be the equivalent of a blastopore, with the divided blastopore of Peripatus as analogy.

But in Calandra with the mid-gut forming from stomodaeum and proctodaeum at relatively advanced stages of development (tracheae are already forming before the proctodaeal component arises) the limits imposed by the gastrula concept have been overstepped; it is impossible, even in principle to reconcile such an embryo with Haeckel's notion of a gastrula, and it is obviously absurd to regard it as representing an extinct gastraea. As Nusbaum and Fulinsky (1909) have shown, we are able to construct a complete series connecting

the extreme cases described by Heymons with species which exhibit Kowalewsky's type of 'gastrula'; but it is evident that as this series progresses all trace of a gastrula is lost.

Earlier writers sought for the gastrula in the blastoderm stage of the embryo, the blastoderm-cells representing the ectoderm, the yolk-cells the endoderm. This view had at least the merit of identifying the gastrula at an early period of development, and not in comparatively advanced embryos as do the above discussed theories. It was based on the belief, then generally held, that the yolk-cells gave rise to the gastral epithelium.[1] With the discovery by Grassi, Kowalewsky, and others that the yolk-cells played no part in mid-gut formation this view was discarded in favour of Kowalewsky's scheme. Yet we have seen that it fails in Calandra.

Other writers (Will, 1888; Hirschler, 1912) even suggest a process of double gastrulation, adopting both the earlier and Kowalewsky's scheme; as Heymons comments, the insect would then recapitulate the gastraea ancestor twice in its own ontogeny.

Heymons himself inclined towards the earlier theory, seeking the gastrula in the blastoderm stage, the yolk-cells being abortive endoderm, while the mid-gut now arose from the ectoderm. Observations on Lepsima (1897 a) and Campodea (1897 b) appeared to confirm this view, for in those species the mid-gut was observed to develop from 'yolk-cells'. Yet judging by Uzel's (1898) account for Campodea there is no real comparison between such cells and the true yolk-cells of other insects, while in Scolopendra true yolk-cells and endoderm occur simultaneously (Heymons, 1901). Lepisma requires reinvestigation. Heymons' position depends on the propriety of homologizing yolk-cells with endoderm; as will appear below, there is no convincing reason for taking such a step.

We conclude then that a gastrula cannot be identified in the embryo of Calandra and probably of many other pterygote insects, without depriving that very useful concept of any meaning that may attach to it.

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[1] It should be observed that Balfour (1880), though he shared this view, justly refrained from identifying a gastrula in the insect embryo; thereby showing the misuse to which the term has been put by less critical writers.

With the question of the gastrula is bound up the thorny problem of identifying the germ-layers. It is not proposed to add yet another discussion to the many on this problem, for they can lead to no verifiable result; it will be more profitable to consider the bearing of the observations on the theory itself.

The indisputable fact of the existence of the germ-layers is the discovery of Pander; their theoretical interpretation is a later development.

In von Baer's great work of 1828–37 they are conceived simply as organs in the making—indeed he gives to the two primary layers the names 'dermal layer' and 'mucous layer', 'because they fully express the significance of these layers'. They are, for him, 'fundamental organs', and he is concerned but little with them until they have become the 'tubes' that form the early embryo. Their significance for him lies in the fact that they are general organs, from which the less general arise. With this conception of their nature the development of Calandra does not conflict.

But with the advent of the evolutionary interpretation of the germ-layers a change in meaning has crept in such that the development of a mid-gut from proctodaeum and stomodaeum, as in Calandra, seems to infringe some basic principle of development. Wherein does the change in meaning lie?

It seems to lie in the notion of the homology of the germ-layers, and in the application thereto of the principle of recapitulation. Haeckel, in giving expression to the doctrine of homologous germ-layers writes (loc. cit., p. 12): 'The metazoa form always two primary germ-layers . . .; their tissues always arise solely from the two primary layers, which have descended from the gastraea to all metazoa, from the simplest sponge to man.' Development is conceived as a process whereby these layers briefly recapitulate their ancestral history, and origin from a common embryonic rudiment becomes a criterion of homology; e.g. homology of the intestine of all metazoa is inferred from its universal origin from endoderm (loc. cit., p. 23). As a corollary, it will be observed, the notion of potentiality has now come in.

It is clear that 'endoderm' has become something more

fundamental than intestine. Haeckel, it is true, looked on the germ-layers as primitive organs (loc. cit., p. 258), for exceptions to the scheme were not contemplated; when however cases were discovered among insects where the intestine did not form from 'endoderm', the need seemed to arise for identifying this germ-layer in the embryo. Heymons (1895 a) then homologized the endoderm with the yolk-cells; Mansour (1927) with certain cells that were said to migrate early in development from the germ-band into the yolk and there degenerate. Reasons for rejecting both these interpretations have already been advanced;[1] it will be more profitable to examine the principle involved.

Experimental methods have shown that development does not proceed in the manner contemplated by Haeckel and his contemporaries. A newt regenerates a normal crystalline lens, not from the epidermis but from its iris; a normal somite and mid-gut wall can arise from presumptive epidermis implanted into a gastrula (Mangold 1925). The course of development of a normal individual from a bud and from an egg are very different; while regeneration may even proceed in disregard of germ-layer specificity (Morgan, 1904). The ontogeny of a metazoan cannot, evidently, be conceived as a process whereby the two primary germ-layers recapitulate their ancestral history and unfold their potentialities. As Spemann (1915) writes: 'an organ is then no longer related through its 'Anlage' with the homologous organ of a nearer or more distant ancestor, but only indirectly, one might say only i d e a l l y , through the general potency of the germ to form the organ, and then its further ability to develop it at the homologous site.'

Homology is an inference from the study of organs. That it is applicable to embryonic organs must be conceded. But when applied to organ-forming regions of an embryo the concept becomes exceedingly vague, and it is doubtful whether any definable meaning then attaches to it. On this point Spemann (1915) writes: 'homologizing is only possible after the formation of 'Anlagen', i.e. at a developmental period when the individual parts of the germ have become differentiated, if not in their

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[1] For a criticism of Mansour's observation see section 7.

outward appearance, at least in their developmental tendency.' Homology of an 'Anlage'—in so far as it is an 'Anlage'—is therefore determined by its fate rather than its origin. If, then, the gastral epithelium of all metazoa is homologous, so are also their 'Anlagen', whatever their mode of formation. But since the name 'endoderm' is applied to that component of the gastrula which is the gut 'Anlage', it is, in principle, not possible to homologize it with yolk-cells of an insect, when these do not actually give rise to the gut.

It is plain that the yolk-cells of insects are a specific embryonic organ, concerned probably with yolk-metabolism. If it is they that are homologous with the endoderm, i.e. mid-gut 'Anlage', of lower metazoa, then clearly the mid-gut 'Anlage' of insects cannot be; and the mid-gut of the adult insect is then also no longer homologous with that of other metazoa.[1] Such are the difficulties which arise when we try to accommodate to the theory facts which were not contemplated in it; and a comparison drawn by Heymons (1901, p. 24) between the cleavage events of myriapods and certain annelids must, as a support for his thesis, now appear vague and unconvincing.

Although the mid-gut 'Anlage' in the embryo of pterygote insects is, surely, homologous with that of other metazoa, since the adult organs are, the term 'endoderm' is not applicable to it; for strictly this term must be reserved for the gut 'Anlage' only when this forms the inner layer of the gastrula.

To make the apparently ectodermal origin of the gut of

[1] Lecaillon (1898) actually sought in this way to avoid conflict with the theory; and Mangold (1925) also suggests that the relationship is not one of pure homology (homogeny) but of homoplasy in Lankester's (1870) sense. Yet similarity of development is not an essential condition of homogeny. Are not the normal and regenerated lenses of the newt's eye 'genetically related, in so far as they have a single representative in a common ancestor', and therefore homogenetic? Lankester's notion of homoplasy is apt to be misunderstood; indeed Spemann himself (loc. cit., p. 79) seems to commit this error when he regards the regenerated lens not as homogenetic but as homoplastic with the normal lens. For when Lankester speaks of 'identical or nearly similar forces, or environments', calling forth similar structures by acting on similar parts of one and the same organism, or on two different organisms, he uses the term 'force' in the evolutionary sense, and not in the physiological sense of 'Entwicklungsmechanik'.

certain insects conform to theory the notion of 'latent endo-
derm' contained in the invaginating fore- and hind-guts, has
been proposed. Heider offered this explanation in 1897, and a
similar idea underlies the suggestion of Nusbaum and Fulinsky
(1909); viz. that the development of the endoderm, which occurs
at the site of stomodaeum and proctodaeum ingrowth, is delayed
till after the formation of these structures, so giving to the
mid-gut the appearance of arising from ectodermal organs. In
so far as 'endoderm' is regarded as simply synonymous with
'mid-gut Anlage' this is, of course, true. But the germ-layer
nomenclature is then retained at the expense of the theory.
The essence of the germ-layer concept is the formation of two
(or three) layers as a very early product of cleavage, the various
organs then differentiating out of these layers. This is the sense
in which Kowalewsky (1871) and Lankester (1873) and Haeckel
(1877) use the term. Hertwig (1906), too, is very explicit on this
point; by germ-layer formation 'is understood the initial
organization of the embryonic cells into individual layers, from
which, then, according to certain rules, all the organs and
tissues take their origin'. It is evident that the notion of 'latent
endoderm' is in direct conflict with the very essence of the theory,
namely, the occurrence of visibly distinguishable layers of cells
in the very early embryo.

We conclude, then, that 'endoderm' does not occur in the
embryo of Calandra.[1] Haeckel homologized the gastral
epithelium of metazoa on the ground of its universal origin from
endoderm; it would seem, rather, that the organ-'Anlage'
is primary, its occurrence as a germ-layer secondary. When
the gastral epithelium arises very early, then endoderm forms;
but when it does not appear till much later, there is no
endoderm.

Recent advances in several branches of biology point to the
need of a more general examination of the germ-layer theory;
but this is quite beyond the scope of the present paper.

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[1] The present observations must not be taken as a support for the para-
doxical assertions that the midgut, in cases where it arises from stomodaeum
and proctodaeum, is ectodermal; it is merely claimed that it cannot be
endodermal.

10. Later Differentiation of the Mesoderm, and Formation of the Epineural Sinus.

About the time the coelomic sacs are beginning to open a narrow ridge develops in the mid-line along the median unsegmented strip of mesoderm; it stretches from just behind the stomodaeum to about the level of the last thoracic segment, and in some embryos even into the abdomen. It arises by the local heaping up of mesoderm cells. Sometimes it projects very prominently, almost like a keel, into the yolk; at other times it is barely recognizable. It may be seen, though not well developed, in fig. 47, Pl. 23; a better example is shown in fig. 75, Pl. 24. Both in the character of its cells (they are rather spindle-shaped, with their axes longitudinal and do not show epithelial arrangement), and in its later development (it forms the blood-cells), it is to be distinguished from the more lateral parts of the median strip. These latter are to be reckoned as part of the 'subsomitic mesoderm', and will be considered below; the median ridge is the 'blood-cell lamella'.

It was first described by Nusbaum in M e l o e, under the name 'Chordastrang'. In German writings it is frequently referred to as the 'Mittelstrang', but as this name is also applied to the entire unsegmented median strip of mesoderm (cf. Hirschler, 1928) confusion will be avoided by using the term 'blood-cell lamella'. A more non-committal name may, however, be required for this very definite structure, because in some insects (e.g. P h y l l o d r o m i a—Nusbaum and Fulinsky, 1906) it is stated to participate in mid-gut formation. In Orthoptera and Dermaptera, according to Heymons, it forms exclusively blood-cells, and with this the present observations on C a l a n d r a agree.

While the coelomic sacs are forming, the nerve-cord is beginning to intrude as a median thickening into the interior of the embryo. The mesoderm between it and the coelomic sacs becomes greatly thickened. These thickenings are segmentally disposed; they fill the entire space between the nerve-cord and the coelomic sacs, and begin now to grow under the coelomic sacs themselves, from which, as yet, they are only indistinctly demarcated. Internally this 'sub-somitic mesoderm' (Eastham)

merges into the lateral parts of the median unsegmented strip, the term 'sub-somitic' being then conveniently applied to the whole. In those segments where appendages develop it merges into the mesoderm of the latter. This mesoderm of the appendage has meanwhile enlarged, and now forms a loose clump of cells completely obliterating its cavity. Reference to figs. 46 and 47, Pl. 23, will make this description clear.

In various insects—Gryllotalpa (Korotneff, 1885), Hydrophilus (Heider, 1889), Donacia (Hirschler, 1909), the mature coelomic sacs are stated to exhibit definite openings into the epineural sinus. MacBride (1914) considers it likely that these openings are the result of the paraffin embedding to which the embryo has been subjected. In Wiesmann's (1926) careful study of Carausius such openings were not seen. In Calandra they do not occur as such in the mature coelomic sac; later, however, when differentiation of the coelomic sac wall begins, with partial break-down of the wall, openings may appear, though usually these are occupied by a mass of mesodermal cells (fig. 75, Pl. 24).

Differentiation of the walls of the coelomic sacs begins early on the third day at a time when the germ-band is just beginning to shorten. From the splanchnic wall arises, as usual, the splanchnic musculature; the fat-body forms from the inferior part of the somatic wall, where it merges into the splanchnic; the external portion of the somatic wall gives rise to the lateral myoblast plate, while the vascular tissue is formed where the splanchnic and somatic walls meet dorso-laterally.

The first indication of differentiation is a loosening up of the inferior wall, the epithelial character of this part being now lost. A coelomic sac in the initial stage of this process is shown in fig. 74, Pl. 24. At a rather later phase of differentiation these cells are to be seen within the coelomic cavity, almost completely obliterating it; from them will develop the fat-body (fig. 75, Pl. 24).

All the coelomic sacs from the labial to the ninth abdominal undergo these changes. The antennary, of course, does not participate.

The further differentiation of the coelomic sacs is accom-

panied by much cell-division, and by enlargement of its parts. The cells of the fat-body begin to acquire their peculiar texture very early, and are soon distinguishable from neighbouring cells by their pale cytoplasm, by their exceptionally big vacuoles and consequently by their large size (figs. 76, 77, Pl. 24). The remainder of the wall of the coelomic sac has lost its epithelial character, the splanchnic muscle rudiment and the lateral myoblast plate being much enlarged and now well-defined. Although the 'Anlage' of the vascular tissue has now become distinguishable, actual cardioblasts cannot yet be recognized.

The subsomitic mesoderm, meantime, has much enlarged, and the fat-body soon begins to spread down over it.

The formation of the epineural sinus begins by a lateral withdrawal of those cells of the subsomitic mesoderm which immediately overlie the nerve-cord, these becoming incorporated into the main segmented masses. In the abdomen, late during the second day, a partial separation of the left and right halves of the mesoderm is seen, and, as the blood-cell lamella is absent here, a space is formed between nerve-cord and yolk. This is actually a precocious formation of epineural sinus (fig. 46, Pl. 23). In the thoracic and gnathal segments the sinus does not appear till well into the third day. Along its whole length the space thus formed expands to the width of the nerve-cord. Three stages in its formation are shown in figs. 76, 77, 78, Pl. 24, all representing sections through the first thoracic segment.

The common description of the epineural sinus arising by shrinkage of the yolk away from the embryo does not hold for Calandra; it is produced by a median withdrawal of mesodermal cells. Nor does it arise, as in Hydrophilus and Donacia as a paired space; it first appears in the mid-line.

11. Alimentary Canal (Later Development).

The development of the alimentary canal has, so far, been described to the stage where it comprises the following parts: (i) a short, blindly ending stomodaeum, which on the third day moves with the head on to the anterior pole of the egg, and so comes to lie horizontally; (ii) a proctodaeum, originally a dorso-ventrally compressed cleft, now become tubular, and, unlike

the stomodaeum, developing a wide opening on to the yolk; (iii) the mid-gut 'Anlage', in the form of two narrow but conspicuous bands of cells, adhering to the splanchnic mesoderm, merging behind into the ventral wall of the open proctodaeum, while anteriorly they converge, just behind the stomodaeum, on to the median sheet of cells from which a small amount of pre-oral mid-gut tissue grows forwards round the stomodaeum.
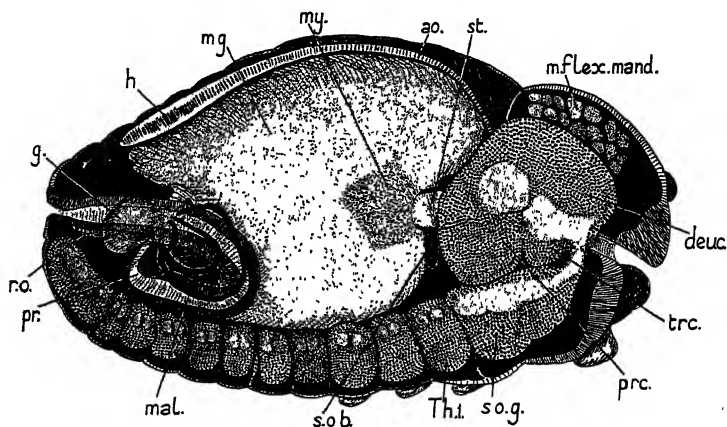
TEXT-FIG. 17.

Advanced embryo, showing internal organs. *ao.*, aorta; *deuc.*, deutocerebrum; *g.*, gonad; *h.*, heart; *mal.*, malpighian tube; *m.flex.mand.*, flexor muscle of mandible; *m.g.*, mid-gut; *my.*, mycetocytes; *pr.*, proctodaeum; *prc.*, protocerebrum; *r.o.*, 'rectal organ'; *s.o.b.*, sub-oesophageal body; *s.o.g.*, sub-oesophageal ganglion; *st.*, stomodaeum; *Th.1.*, first thoracic ganglion; *trc.*, tritocerebrum.

A. The Mid-gut.—With the movement of the head on to the front pole of the egg, during the third day, the yolk becomes pushed backwards. Owing to the development of the great head ganglia and the enlargement of the head muscles, the yolk, now much diminished in quantity, becomes pushed back well into the thorax (Text-fig. 17).

At the time the coelomic sacs are beginning to differentiate the associated mid-gut cells again start to multiply. They

spread at first downwards under the yolk; soon also, as the lateral body-walls enlarge, upwards, till on the fourth day the yolk becomes completely enclosed. That portion of the yolk which at an earlier stage lay pre-orally, now lies above the stomodaeum. The pre-oral mid-gut cells therefore form that portion of the intestinal wall which lies immediately above the stomodaeum; this will be understood from fig. 79, Pl. 24, where the cells still form a little clump, and have not yet spread as an epithelium over the yolk.

Throughout this period the mid-gut cells are characterized by their hyaline and slightly vacuolated cytoplasm. At the time the spreading begins they are usually packed several cells deep; gradually, however, they separate to form the simple epithelium of the larva. This is accompanied by a large increase in their volume. Scattered in considerable numbers among these large cells are smaller ones, with nuclei less than half the diameter of those of the functional mid-gut cells; they are the 'replacing cells' whose further development is described in our previous paper (Murray and Tiegs, 1935). They are to be seen in fig. 71, Pl. 24, and are already recognizable in the section drawn in fig. 78, Pl. 24.

As the mid-gut cells gradually spread round the yolk the associated layer of splanchnic mesoderm, in which all trace of segmentation is now lost, spreads with them, and so ensheaths the mid-gut epithelium. A clear line of demarcation is always visible between the two layers. Segments anterior to the labial do not contribute splanchnic mesoderm to the intestinal wall, the mesodermal sheath investing its anterior end being derived from cells that move forwards from the labial segment. This does not, actually, involve any extensive migration of cells, for it is at this period that the gnathal segments are becoming reduced in size and only the most anterior tip of the mid-gut lies before the labial segment.

Although most of the splanchnic wall of the mid-gut thus comes from the coelomic sacs, an exception must be made for a portion which lies just dorsally to its connexion with the proctodaeum. If fig. 66, Pl. 23, be examined a thin sheath of cells will be seen closely investing the yolk. These cells are

derived from the mesoderm that has migrated forwards from the eleventh segment, but most of which is used in the formation of the investing sheaths of the proctodaeum and malpighian tubes (see below 11 *C*). With the completion of shortening of the germ-band the position of this membrane, which has till now faced downwards, becomes inverted. But owing to withdrawal of yolk from the hind\'er end of the embryo the membrane becomes drawn forwards with it, and so forms the splanchnic sheath for the postero-dorsal portion of the mid-gut. Reference to Text-fig. 17 will make this description clear.

Differentiation of the splanchnic coat into circular and longitudinal muscle layers, and probably also into serosa, occurs during the fourth day.

During the last day the mid-gut assumes its definitive form. At about the end of the third day, when the embryo has just completed its shortening, the yolk still extends backwards, dorsally, well towards the hinder end of the embryo, though ventrally it is displaced forwards by the enlarging proctodaeum (cf. the more advanced embryo in Text-fig. 17). But after enclosure of the yolk has been completed it undergoes a rapid absorption. The mid-gut at the same time alters its form, being converted from a large ungainly sac into a more elongate but still spacious organ. In front it is wide; the hinder end, however, particularly where it merges into the proctodaeum, has become quite narrow. The characteristic coiling, previously described (Murray and Tiegs, 1935) appears during these events.

The intestinal caeca, which are confined to the narrow hinder portion of the mid-gut arise, in the latter half of the fourth day, as solid outgrowths from the mid-gut epithelium (fig. 71, Pl. 24); the intercellular lumen forms later.

B. The Stomodaeum.—By the end of the second day the stomodaeum has become cylindrical with a narrow lumen, and projects prominently into the yolk.

At about this period cells begin to separate off from the blind end of the stomodaeum and migrate in a long string into the yolk (fig. 64, Pl. 23). They have already been referred to by Mansour (1927). Their fate is described below (11 *E*).

With the beginning of shortening of the embryo the enlarging head, as already described, moves on to the anterior pole of the egg. This brings the stomodaeum into a horizontal position (fig. 79, Pl. 24). The yolk, at the same time, becomes pressed backwards, as the great head ganglia develop. This is attended by a great elongation of the stomodaeum, due to intense proliferation of its cells. In some embryos it even bulges deeply into the yolk.

Active proliferation of its cells continues till about the end of the third day, the stomodaeum being now a thick-walled blindly ending tube, composed of densely packed cylindrical cells. The further enlargement which occurs in the last day is due, not to multiplication of cells, for mitosis is not seen, but to a change in the form of the cells, which become cubical or even flattened. The hind end of the stomodaeum at the same time expands to form the crop.

During the fourth day the tip of the stomodaeum becomes reduced to an exceedingly thin membrane. Its condition at about the end of the third day is shown in fig. 79, Pl. 24. Many of its cells now undergo transformation into mycetocytes (fig. 101, Pl. 25) which then become incorporated into the main mass of mycetocytes which is accumulating round the stomodaeum at this period (v. below 11 E). Only a few cells remain behind to form a partition between the yolk and the lumen of the stomodaeum. As the end of the stomodaeum expands to form the crop this membrane becomes stretched to an almost imperceptible fineness. It persists thus up to about the time of emergence.

The muscle-coat of the stomodaeum is derived chiefly from the pre-oral mesoderm, though the post-oral (i.e. premandibular) also contributes a part. In Pieris Eastham (1930 a) finds that the pre-oral mesoderm alone is concerned; but Forficula, according to Heymons' account, resembles Calandra. The two masses of mesoderm appear in the section shown in fig. 79, Pl. 24, the pre-oral being much the more conspicuous. In fig. 85, Pl. 25, which represents a horizontal section along an embryo at the beginning of shortening, the origin of this mesoderm from the premandibular segment is seen; reference to Text-fig. 12

will make the relationship clear. As development progresses, the two masses form a complete investment for the stomodaeum (fig. 89, Pl. 25). Differentiation into circular and longitudinal muscle-coats occurs during the fourth day; the small oesophageal dilators (figs. 116, 118, Pl. 26) also arise from it.

C. The Proctodaeum.—In the foregoing account the proctodaeum has attained the condition of a short tubular organ, opening internally on to the yolk, its thick ventral wall being continuous with the mid-gut 'Anlage' that has arisen as a pair of band-like outgrowths from it.

The internal opening of the proctodaeum survives till early in the third day. Closure begins by the formation, immediately in front of the opening of the malpighian tubes, of a transverse partition derived entirely from proctodaeal cells. By the time the germ-band has completed its shortening a second layer has been formed, from the mid-gut epithelium.

Meanwhile, the proctodaeum elongates rapidly owing to much division of its cells. During this phase of rapid growth its wall is a thick epithelium of narrow columnar cells. Differentiation is first seen early on the fourth day. The intense cell-division has now ceased; the cells begin to enlarge, particularly along the floor of the hinder (rectal) portion, while at the same time the organ as a whole elongates and begins, in consequence, its characteristic coiling (Text-fig. 17). The differentiation continues during the fourth day.

Communication between mid-gut and hind-gut is re-established late on the fourth day. In fig. 73, Pl. 24, is shown a sagittal section of the intestine at the junction of the two, immediately prior to break-down of the partition. From the appearance of the cells it seems probable that they become withdrawn into the intestinal wall.

Both the musculature of the hind-gut, and the peculiar organ referred to as 'rectal-organ' (Text-fig. 17) in our previous paper (Murray and Tiegs, 1935) are derived from the large mass of mesoderm cells situated behind the genital rudiment. As the proctodaeum becomes tubular and elongates these cells move forwards, and envelop it along its whole length. Into this mass before it has yet become organized into an investing sheath

for the proctodaeum, the three pairs of malpighian tubes grow.

Some of the mesodermal cells now show a tendency to apply themselves to the walls of the latter (fig. 67 A, Pl. 24), others form a sheath for the proctodaeum itself. With the formation of the haemocoele this portion of the embryo becomes 'loosened up'. We can then clearly distinguish the following (fig. 66, Pl. 24): (i) a thick mesoderm sheath investing the rectum, and giving rise to the rectal muscle-coats, the 'rectal-organ' and the investing serosa; (ii) a thin sheath ('serosa') investing each malpighian tube; (iii) the thin sheath, alluded to above (section 11 A), from which will arise part of the splanchnic coat of the mid-gut.

Differentiation of the 'rectal-organ' occurs surprisingly early, namely at the time when the germ-band has just completed its shortening. The rectal musculature does not differentiate till the end of the fourth day.

D. The Yolk.—Division of the yolk-cells, described in section 3 B, does not occur beyond the blastoderm period. With the formation of the germ-band the nests of yolk-cells give place to single cells scattered, as a syncytium, through the yolk. By about the middle of the second day the syncytium is beginning to break up into parts, till eventually each nucleus becomes the centre of a small yolk-sphere. At first multinuclear masses occur (figs. 51, 52, 55, Pl. 22), but at about the end of the second day have usually become resolved into single cells (fig. 64, Pl. 22).

During the third day, when the yolk is being rapidly absorbed, many of these yolk-nuclei are seen in stages of degeneration or disintegration. Thereafter the yolk gradually becomes a disorganized mass of yolk- and fat-globules, nuclei, and fragments of cytoplasm with only seldom a sign of intact cells (figs. 79, Pl. 24; fig. 115, Pl. 26). Remains of the yolk survive till after emergence of the larva.

E. The Symbiotic (?) Bacteria and the Mycetoma.—The peculiar relation that exists between a bacterial organism and certain tissues of Calandra oryzae has been recorded in several recent papers (Pierantoni, 1927; Buchner, 1930; Mansour, 1930; Murray and Tiegs, 1935). The manner

of infection of the sexual organs is described in section 4; here we describe the relationship to the gut.

At the end of the second day isolated clumps of bacteria are occasionally encountered in the yolk (fig. 64, Pl. 23). To these now become added great masses of bacteria that arise from the disruption of mycetocytes that migrate into the yolk from various parts of the developing gut-wall.

The first to appear are a group of cells which become liberated from the tip of the stomodaeum, and migrate as a long string of cells into the yolk (fig. 64, Pl. 23). At the time of their first appearance they are not visibly distinguishable from the adjacent stomodaeal cells. But in more advanced embryos, when the germ-band is beginning to shorten, they have completely changed their appearance, and are now seen to be packed with a felt-work of bacteria. Many of these mycetocytes disrupt, great clumps of bacteria with nuclei scattered among them thus coming to lie freely in the yolk.

To this bacterial mass are now added cells, already observed by Mansour (1927), which migrate from the walls of the developing mid-gut. From the mid-gut 'Anlage' as it spreads upwards over the yolk, and particularly from its dorsal edge, sheets of cells spread inwards into the yolk (fig. 77, Pl. 24). In appearance they are indistinguishable from normal mid-gut cells. Within the yolk they enlarge and become converted into mycetocytes (figs. 77, Pl. 24; fig. 115, Pl. 26). Many remain intact; but mostly they disrupt, shedding their bacterial content into the yolk, where it forms conspicuous masses of a dense feltwork of bacteria, lying amidst the yolk.

Formation of the mycetoma begins by an accumulation of the intact mycetocytes round the blind end of the stomodaeum (fig. 79, Pl. 24; Text-fig. 17). As this mass enlarges, mycetocytes, that arise by transformation of cells from the blind end of the stomodaeum, become added to it; indeed, most of the cells of the immediately adjacent stomodaeal wall seem to undergo this fate (fig. 101, Pl. 25). Eventually, as Mansour observed, the whole forces its way out from the yolk and becomes lodged as the conspicuous mycetoma just below the crop (figs. 69, 70, Pl. 24; Text-fig. 18).

A review of the literature on the adaptation of embryonic events to symbiosis is beyond the scope of this paper. Buchner's comprehensive work (1930) may be referred to. Nothing comparable with the remarkable events observed in Calandra oryzae seems to have been hitherto recorded.

12. The Salivary Glands.

These are associated not with the labium but with the base of the maxillae, on the inner aspect of which they open. They arise late on the third day as long tubular invaginations of the ectoderm, and extend back as far as the mycetoma, where they coil a little. Fig. 79, Pl. 24, shows an early stage of development.

13. The Malpighian Tubes.

In the larva three pairs of malpighian tubes occur. They open into the anterior end of the hind-gut, and doubling back on themselves, are attached at their blind ends to the rectum. One pair is short; the other two much longer, one of these being particularly long and extending to the anterior end of the midgut before bending back (for illustration see Murray and Tiegs (1935), Text-fig. 1).

One pair much precedes the other two in time of development. The first two tubes arise in the latter half of the second day as a pair of invaginations from the floor of the proctodaeum (fig. 62, Pl. 23). In some exceptional cases they are distinguishable at a time when the hinder end of the germ-band is just bending down into the yolk (fig. 60, Pl. 23); but usually they appear rather later (fig. 61, Pl. 23). Although they arise so early their further development is retarded till about the end of the second day when they begin to elongate. They grow backwards alongside the proctodaeum, and acquire an investing sheath of mesoderm in the manner already described (section 11 C).

No indication of the remaining malpighian tubes has been seen in any embryo till early in the third day, at a time when the proctodaeum has become converted into a cylindrical tube. As the tubular form develops the openings of the first pair of malpighian tubes are carried on to the sides of the proctodaeum (fig. 67 B, Pl. 24). The remaining tubes arise, like the first, as

hollow outgrowths of the proctodaeal wall, a little behind and dorsal to the first pair (fig. 67 A, Pl. 24). Like the first they grow backwards alongside the proctodaeum, so that three pairs appear simultaneously in transverse sections through this region, of which the first pair is distinguished by its greater size (fig. 66, Pl. 23).

During the third day active cell-division causes much elongation of the tubes. Late on the third day the proctodaeum begins to bend, and with it the malpighian tubes (Text-fig. 17). A phase of differentiation, unaccompanied by cell-division, sets in, and the cells, hitherto rather columnar and loosely packed, become considerably flattened, while the whole tube becomes thinner and develops a more compact texture, the duct itself becoming better defined. Simultaneously the tubes lengthen, particularly the two larger pairs; the blind hinder end retains connexion with the wall of the proctodaeum, while the middle portion is pressed forwards in the haemocoele, and so adopts the peculiar form seen in the larva.

Already at this early period two kinds of cells—with small and with large nuclei—are distinguishable in the wall of the malpighian tube (fig. 72, Pl. 24); it is very probable that they are the imaginal and larval cells respectively. The ultimate enormous disproportion in their size appears only later during the growth of the larva (see Murray and Tiegs, 1935).

14. THE SUB-OESOPHAGEAL BODIES.[1]

The organs referred to by this name were first described by Wheeler in 1893 from the embryo of X i p h i d i u m and have since been seen in a variety of species. They are stated to be more conspicuous in the embryo than in the larva, and do not survive into the imago. In this latter respect C a l a n d r a is an exception.

[1] The occurrence of this organ in the larva was overlooked in our previous paper. It is found throughout the larval period as a small paired flattened cell-mass, adhering to the underside, and in fact, incorporated into the body of, the mycetoma. Here its cells grow markedly in size. It survives the metamorphosis, occurring in the imago as a pair of inconspicuous bodies at the hinder end of the gizzard. The number of its cells has become reduced to about 9–12. They are clumped into several masses in which cell-boundaries are hardly recognizable. Their appearance suggests nephrocytes.

Embryologically the bodies are of interest because two distinct methods of development have been assigned to them. They are generally held to be derivatives of the premandibular (intercalary) mesoderm—Orthoptera (Wheeler, Heymons, Wiesmann); Isoptera (Strindberg), Pieris (Eastham), Wiesmann stating that in C a r a u s i u s they arise quite obviously from the premandibular somite.[1] Yet according to Nusbaum and Fulinsky (Orthoptera), Hirschler (D o n a c i a), Schwangart, Hirschler, and Johannsen (various Lepidoptera) they are midgut derivatives. Whether there is uniformity in their development must, for the time, remain uncertain; present observations on C a l a n d r a, however, offer a possible explanation for this difference of opinion, for though the bodies arise from the mesoderm they become secondarily part of the mid-gut wall and have the appearance of arising from the latter.

They first become clearly recognizable in embryos in which shortening is just beginning, appearing as a pair of rounded bodies, often enclosing a cavity, and located at the anterior angle of the mandible just behind the stomodaeum, i.e. in a region corresponding to the intercalary segment. This is readily seen in the horizontal section shown in fig. 85, Pl. 25. Externally they abut on the tritocerebral ganglion; internally they are in contact with the yolk (fig. 84, Pl. 25). Although a few mid-gut cells already appear in association with them, they are not actually a part of a definite mid-gut wall; in later embryos, however, when a complete mid-gut epithelium has become stablished, they appear as part of the mid-gut wall, being situated as a pair of very conspicuous bodies immediately ventro-lateral to the base of the oesophagus (fig. 79, Pl. 24), and giving the appearance of having developed as outgrowths from the mid-gut. The cells throughout this period are already distinguishable by their large size, and by the paleness and characteristic faint granulation of their cytoplasm.

It remains then to determine whether they are derivatives of the mid-gut cells, or whether they come from the mesoderm. The latter proves to be the case. This is well seen in fig. 86,

[1] In Roonwal's recent work (Phil. Trans. Roy. Soc. B. 227. 1937) they are found to come from the mandibular mesoderm. (L o c u s t a.)

Pl. 25, which represents part of a section of a transversely cut, rather earlier embryo, taken at about the same level as that shown in fig. 84, Pl. 25, i.e. through the tritocerebral ganglion. It will be seen that the cells of the sub-oesophageal body, already distinguishable by their size and peculiar texture, occupy the position of mesoderm cells. A definite somite, as described by Wiesmann for Carausius, they do not form; yet at a rather later phase, as shown in figs. 84, 85, Pl. 25, they show often a remarkable resemblance to coelomic sacs.

In criticism of Hirschler's (1907, 1928) contention that the sub-oesophageal body arises from the mid-gut and not from the intercalary mesoderm, it should be observed that, according to his observations on Donacia, this mesoderm, in the form of a pair of large coelomic sacs, is utilized in the formation of the cephalic aorta; yet general experience shows the aorta to arise from the antennary coelom (which is said to be absent in Donacia), or at any rate, from antennary mesoderm, while an intercalary coelom occurs only in the most primitive insects, and then as a vestige at most. It seems very probable that the Donacia embryo has been misinterpreted; an examination of figs. 83, 84, 85, and 87, Pl. 25, will show how easily antennary and intercalary mesoderm can be confused.

In later embryos of Calandra the sub-oesophageal bodies again lose connexion with the mid-gut. This occurs during the fourth day. As the mycetocytes begin to move through the gut-wall they carry the sub-oesophageal bodies before them. The cells of the latter begin to spread out on the ventro-lateral parts of the mycetoma, into the contours of which they become incorporated (figs. 69, 70, Pl. 24). Histologically the two tissues remain distinguishable.

A formation of blood-cells from the sub-oesophageal bodies, as described for some species, does not occur in Calandra.

15. The Corpora Allata.[1]

According to all who have investigated the matter, these bodies are ectodermal. Heymons (1895) in Forficula was the

[1] These organs were not referred to in our previous account. In the newly hatched larva they occur as a pair of compact ovoidal bodies, with radial

first to describe their formation; from his account they appear to arise as ingrowths from the anterior angle of the maxilla, a pair of rounded bodies becoming constricted off and eventually finding their way, by the aid of the maxillary tentoria, to the antennary coelomic sacs, to the lower ends of which they then attach themselves. This appears to be the case also in B a c i l l u s r o s i i (Heymons, 1897 c), C h a l i c o d o m a (Carrière and Bürger, 1898), F o r m i c a (Strindberg, 1913), A p i s (Nelson, 1915), and P i e r i s (Eastham, 1930 a).

There seems to be no reason for doubting these accounts; yet in C a l a n d r a the bodies develop quite differently. They arise from the antennary segment, and though they form in intimate association with the tentorium, they are, as far as could be ascertained, purely a differentiation of the ventral wall of the antennary coelomic sac, i.e. they are mesodermal.

By the beginning of the third day, when the germ-band has started to shorten, the antennary coelomic sac (section 8) has become clearly defined as a small vesicle which may project a little into the cavity of the antenna, though the major part of

cell arrangement, at the anterior ventral surface of the brain, a little behind the circum-oesophageal nerve-strands. To the brain they are joined by a large tracheal vessel that enters the latter; with the cephalic aorta they are connected by membrane (remnant of the antennary coelomic sac), which itself forms a delicate investment for the corpora. Their position is shown in Text-fig. 18. A nervous connexion with the sympathetic could not be detected.

During larval life the bodies enlarge considerably but remain in position under the brain. But already in the early pupa they have become drawn up to their definitive position in the dorso-lateral wall of the oesophagus, to the side of and just behind, the hypocerebral ganglion. The tracheal connexion with the brain is still present. Nabert (1913) has already described a similar movement of the corpora allata during metamorphosis in a number of species.

In the young larva they form a small solid ball of cells with deeply staining peripheral nuclei. During the larval period the cells become about doubled in number and also enlarge. The middle of the organ presents a granular eosinophile protoplasm (ground substance of gland); cell-walls are recognizable only at the outer nucleus-bearing periphery. At the onset of metamorphosis the ground substance often presents a peculiar hyaline appearance, and may exhibit nuclei. In aged adults (5–6 weeks old) the bodies appear markedly swollen, and the nuclei much enlarged.

it lies behind the appendage (figs. 83, 86, 87, Pl. 25). Two events now occur which bring about a backward movement of the coelomic sac—the development of the brain and of the tentorium. As the brain gradually develops by thickening of the ectoderm in front of the antenna and as it enlarges and grows backwards, it pushes the underlying antennary coelomic sac far back into the cavity of the head. The development of the tentorium (q.v.) keeps pace with these events. We are concerned here only with the antennary component of the tentorium. This grows back as a tubular ingrowth of the ectoderm from just behind the antenna, to meet eventually a similar ingrowth from the hinder angle of the maxilla. As the ingrowth from the antenna develops, a loose connexion is observed between it and the adjacent coelomic sac, due to the outgrowth of fine protoplasmic strands from cells in the ventral wall of the latter. Fig. 79, Pl. 24 and fig. 88, Pl. 25, will make this description clearer. They are drawn from longitudinal sections of a single embryo, in which the plane of section is tilted considerably from the sagittal plane. In fig. 79, Pl. 24, only the lower part of the coelomic sac appears, but its relation to the sub-oesophageal body (i.e. premandibular mesoderm) is shown. In fig. 88, Pl. 25, from the opposite side of the same embryo the connexion with the tentorium is seen, as well as its primitive relation to the brain.

In the meantime the coelomic sacs have considerably enlarged, mitosis of their cells being frequently seen. The cells are distinguishable from those of the adjacent tentorium by the rather deeper staining of their nuclei.

Formation of the corpora allata begins with a thickening of the ventral wall of the coelomic sac (figs. 88, 89, Pl. 25). The cells of the thickening present the distinguishing features of the remaining cells of the sac wall, there being no evidence for a migration of ectodermal cells of the tentorium over to it. The section shown in fig. 89, Pl. 25, it should be explained, passes just behind the antenna, the posterior wall of the latter being partially included in the section; the large mass of cells between it and the transected tentorium being also tentorial cells cut along their line of ingrowth from the base of the antenna.

A later stage of development is shown in fig. 90, Pl. 25.

Only a portion of the coelomic sac, which is now much enlarged, is shown in the drawing. Its walls have become thinner, and the corpus allatum sharply defined.

A rather later stage is shown in fig. 105, Pl. 25 and fig. 116, Pl. 26. While the coelomic sac has greatly elongated and thinned out, the corpus allatum has become more distinct still, while the tracheal vessel, referred to in the appended footnote, p. 238, has come into association with it.

In fig. 91, Pl. 25, from an embryo in which shortening is not quite complete, the body has become invested in a sheath; and although a connexion of the coelomic sac with the tentorium survives, the body itself is no longer connected with the tentorium.

Finally during the fourth day the cells differentiate; they enlarge, and adopt a radial arrangement, their cytoplasm becoming more eosinophil, and in this way occur in the newly emerged larva.

Since these conclusions differ radically from those of all other workers, it is desirable to make some comments on their objective validity. An origin from the base of the maxilla is excluded by the facts (i) that the body may be observed in process of differentiation from the walls of the coelomic sac, (ii) that in some embryos the body is in an advanced state of development before the tentorial ingrowth from the antenna has met that from the maxilla, the participation of the latter in transferring the corpora allata to the coelomic sacs, as described by Heymons for Forficula being thereby excluded. The chief trouble in the present work has been the possibility of migration of cells on to the coelomic sac from the adjacent antennary ingrowth, which would render them ectodermal. In the absence of marking experiments reliance has been placed only on the visible, though often not very well defined distinction in the appearance of the two types of cell; throughout their development the cells of the corpora allata resemble those of the coelomic sac and not of the tentorium.

16. The Blood-vascular System, and Related Structures.

A. The Haemocoele.—This arises late on the third day in embryos in which shortening has begun, the first evidence of

its formation being the appearance of the epineural sinus. Its development is described in section 10.

At about the end of the third day the fat-body, now much enlarged, separates from the adjacent lateral walls of the mid-gut, the lateral blood sinus, merging below into the epineural sinus, thus arising (fig. 78, Pl. 24; fig. 115, Pl. 26). When the lateral sinuses eventually merge into one another above the gut, the latter becomes entirely contained within that space.

In the meantime, by the separation of the nerve-cord from the epidermis, the latter also becomes contained within the haemocoele (figs. 77, 78, Pl. 24; fig. 115, Pl. 26).

By separation of the proctodaeum from the adjacent fat-body the haemocoele spreads into the terminal body segments (fig. 66, Pl. 23).

In the head also a prominent space appears. It arises after shortening of the embryo has begun, and owes its formation to a withdrawal of the yolk from the head region, due to its gradual absorption and its enclosure within the mid-gut. The space so formed, which is continuous behind with the haemocoele of the thorax, is partly occupied by the enlarging antennary coelom (fig. 89, Pl. 25); but with the complete withdrawal of the yolk, and with the subsequent diminution in size of the antennary coelom, it becomes greatly enlarged and accommodates the brain (figs. 105, Pl. 25; fig. 116, Pl. 26).

B. The Blood.—The blood-cells are derived entirely from the 'blood-cell lamella'—the median unsegmented ridge of mesoderm cells that extends from just behind the stomodaeum to the level of the last thoracic, or even, occasionally, second or third abdominal segment (section 10).

At the time the embryo is beginning to shorten, these cells, hitherto spindle-shaped and exhibiting marked longitudinal polarity, become rounded, and by the time the epineural sinus has formed, have acquired the peculiar histological features—clear hyaline and often slightly vacuolated cytoplasm—by which they can usually be identified (cf. fig. 77, Pl. 24). Although in Calandra they arise only in the anterior half of the embryo they may, late in the third day, be encountered floating at random anywhere in the epineural sinus.

No other source of blood-cells, whether from the heart or from the sub-oesophageal body, could be detected.

According to Nusbaum and Fulinsky (Phyllodromia), Hirschler (Donacia), and Philiptschenko (Isotoma) mid-gut cells arise from the same primitive 'Mittelstrang' which is the source of the blood-cells; there is, however, no evidence for this in Calandra, in which respect the present observations agree with those of Korotneff (Gryllotalpa), Heymons (Orthoptera and Dermaptera), and Eastham (Pieris).

C. The Fat-body.—This occurs, in Calandra, in two distinct parts: (i) a bulky visceral portion occupying most of the haemocoele, and in later larvae almost obliterating it; (ii) a comparatively inconspicuous parietal zone of smaller less vacuolated cells, lying just under the epidermis and external to the muscles (Murray and Tiegs, 1935).

The main portion of the fat-body is derived from the inferior wall of the coelomic sacs from the labial to the ninth abdominal segment. The early stages are described in section 10. After separating from the wall of the coelomic sac the cells lose their capacity for deep staining and become more and more highly vacuolated. The fat-cells thus rapidly enlarge, and spread towards, but not into the epineural sinus, while laterally the body extends upwards in the haemocoele as the walls of the embryo encircle the yolk. Although fat-cells arise in the labial segment they do not become included in the head. In advanced embryos the fat-cells clump together into multinucleated masses.

The parietal fat-body arises independently of the visceral, and seems to be derived from cells originating in the external wall of the coelomic sac. At about the end of the third day, when the development of the visceral fat-body is already advanced, a layer of rather large scattered cells is seen between the epidermis and the lateral masses of myoblasts. They do not appear to arise from the adjacent ectoderm, but seem rather to be small remnants of the cells of the external coelomic sac-wall, that have not been utilized in muscle formation. A few parietal fat-cells are seen in figs. 112, 115, Pl. 26.

D. The Dorsal Blood-vessel.—This comprises (i) the heart proper, with four ostia and four main alary muscles,

(ii) the aorta, (iii) the cephalic aorta. The heart and aorta will be considered first.

These develop from the dorso-lateral walls of the coelomic sacs. Every such sac, from the ninth abdominal forwards to the labial, is concerned; but as far as could be determined the gnathal mesoderm anterior to the labial does not contribute to their formation.

Within the intact coelomic sac it is impossible to identify the limits of the cardiac tissue for, unlike some insects, not even the cardioblasts are distinguishable till much later; in D o n a c i a Hirschler could detect cardioblasts in the mature coelomic sac, while in F o r f i c u l a Heymons observed a conspicuous paracardial-cell 'Anlage'.

By the time the embryo has begun to shorten, concrescence of the coelomic sacs is well advanced. The cardiac 'Anlage' now becomes stretched to a thin membrane between the splanchnic mesoderm and the ectoderm, abutting on the yolk above, and covering the fat-cells below (fig. 76, Pl. 24), sections taken along the lateral body-wall revealing it as a columnar epithelium in which traces of the initial segmentation have still survived (fig. 103, Pl. 25).

Cardioblasts first become distinguishable in the anterior segments, appearing as a succession of enlarged cells immediately adjacent to the ectoderm (fig. 77, Pl. 24, from an embryo at about the stage of Text-fig. 13).

In the abdomen the development of the alary muscles and the nephrocytes (paracardial tissue) introduces a complexity. A section through the 'Anlage' at the level of the first alary muscle, just before differentiation of the cardioblasts, is shown in fig. 108, Pl. 25. The conspicuous mass of mesoderm adjacent to the ectoderm is only partly concerned with heart formation, some of its cells being somatic myoblasts. An early stage in its differentiation into cardioblasts, alary muscle, and myoblasts is seen in fig. 109, Pl. 25. A more advanced stage, from an embryo that has nearly completed its shortening, is seen in fig. 110, Pl. 25; cardioblast, alary muscle, pericardial, and paracardial rudiments are now all apparent.

With the spreading of the lateral body-wall upwards over the

sides of the yolk the two heart rudiments (Anlagen) are carried on to the dorsal surface of the embryo and there meet. Closure occurs early on the fourth day, appearing first at the anterior, later at the posterior end, and most delayed in the mid-region.

The formation of a closed tube is attended by a change in shape of the cardioblasts. Till now the cardioblasts on each side have formed a row of very large flattened cells placed, like a row of books, with their flat surfaces apposed, so that in lateral view they give a peculiar pallisade appearance (fig. 113, Pl. 26) while the true width of the cell is visible only in transverse section (fig. 111, Pl. 26). But as the tube develops they change into long curved cells (fig. 112, Pl. 26).

The ostia are recognizable as clefts between the cardioblasts (fig. 113, Pl. 26).

Throughout this period of development the heart tissue remains loosely connected by a 'mesentery' with the splanchnic mesoderm that invests the mid-gut. This is best seen at the hinder end of the embryo where the gut shrinks well away from the body-wall. In Hirschler's description for D o n a c i a the cavity of the heart, incompletely closed below, is said to be temporarily continuous through the 'mesentery' with a narrow sinus surrounding the proctodaeum, and in C a r a u s i u s Wiesmann found a well-developed sinus around the mid-gut. There are indications in C a l a n d r a also of such a sinus at the posterior end of the mid-gut, but it is, at best, only a narrow ill-defined cleft (fig. 111, Pl. 26). In later embryos the last remnant of 'mesentery' disappears (fig. 112, Pl. 26).

Differentiation of the various associated cells occurs very late. In fig. 114, Pl. 26, from an advanced embryo the network of pericardial (adventitial) cells, as well as the clumps of nephrocytes is seen; it will be observed that the latter have remained segmental.

Blood lacunae such as occur in various Orthoptera (Korotneff, 1885; Heymons, 1895) prior to formation of the heart, are not present in C a l a n d r a.

The cephalic aorta is formed from the antennary coelomic sacs. The earlier development of these sacs is described in section 15.

While the corpora allata are differentiating from their lower ends the walls of the sacs are becoming thinner and thinner, the small compact vesicles becoming converted into long delicate sacs which extend to the side of the stomodaeum underneath the protocerebrum up towards the most anterior cardioblasts.

In backwardly sloping, rather than transverse, sections it is possible to include the coelomic sac for its whole length within a single section. Fig. 105, Pl. 25, is drawn from such a section; at its lower end the coelomic sac, now very attenuated, is attached to the tentorium, and with it is connected the corpus allatum, while at its upper end it has come into association with the most anterior cardioblasts of the same side.

A later stage of development is shown in fig. 106, Pl. 25. The two rows of cardioblasts have united to form a tube, here cut longitudinally, for it is bending down over the anterior end of the mid-gut (cf. Text-fig. 18); the two coelomic sacs have become intimately connected with the end of the aorta, and it will be observed that while their internal walls are continuous with the aorta itself, their external walls fuse with the adventitia. In the more advanced stage shown in fig. 107, Pl. 25, the internal wall of the coelomic sac has assumed the structure of the wall of the aorta.

It will be evident, and this can be confirmed in appropriately cut sections, that the cephalic aorta has arisen by the union of two grooves on the internal dorsal faces of the sacs. The aorta thus formed passes above the ventricular ganglion; but at the level of the hypocerebral ganglion closure occurs in such a way that the ganglion itself becomes enveloped by it (Text-fig. 18).

The anterior opening of the cephalic aorta is a little in front of the hypocerebral ganglion. The adventitial layer, in addition to forming the very delicate investment of the aorta, also spreads downwards in this region as a thin membranous hood, attached below on either side to the tentorium, and having the corpora allata still appended to it. In addition to attachment to the tentorium it is also supported by a long and exceedingly fine filament which is inserted in front on to the head-capsule. The whole organ is shown in Text-fig. 18. It will be evident, in comparison with fig. 105, Pl. 25; fig. 116, Pl. 26,

that it is the ventral part of the coelomic sac, the aorta forming from only the dorsal portion.

The origin of the cephalic aorta from the antennary coelom accords with general experience (Forficula Heymons;
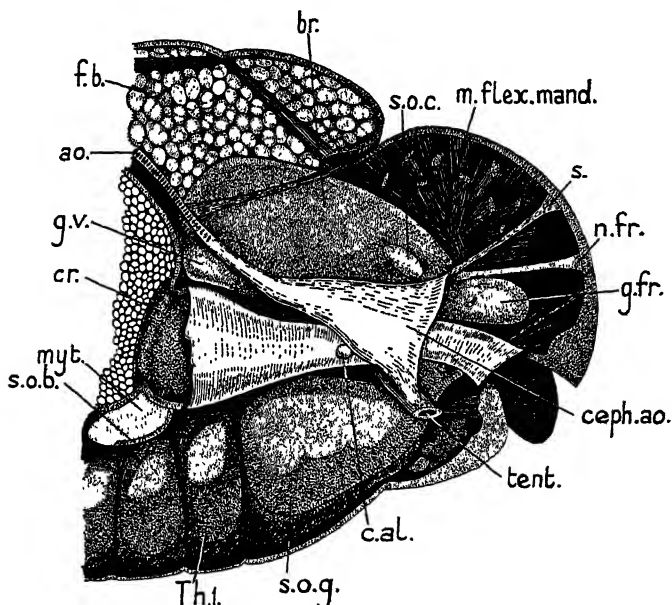
TEXT-FIG. 18.

Anterior end of very advanced embryo, drawn as a dissected object. Lettering as in Text-fig. 17; additional: *br.*, brain; *c.al.*, corpus allatum; *ceph.ao.*, 'hood' of cephalic aorta; *cr.*, crop, separated by membranous partition from yolk of mid-gut; *f.b.*, fat-body; *g.v.*, ventricular ganglion; *g.fr.*, frontal ganglion; *myt.*, mycetoma; *n.fr.*, frontal nerve; *s.*, suspension for cephalic aorta; *s.o.c.*, supra-oesophageal commissure; *tent.*, tentorium.

Eutermes and Formica Strindberg; Apis Nelson; Carausius Wiesmann); Hirschler's contention that it arises from an intercalary coelomic sac is discussed in section 14.

17. THE EPIDERMIS AND SIMPLE DERIVATIVES.

A. The Epidermis.—To the end of the third day this is a closely packed columnar epithelium. After complete enclosure

of the yolk differentiation begins, the cells becoming cubical and chitinizing on their outer face. There is no evidence for a special embryonic cuticle.

The structure of the thoracic appendages of the embryo is shown in figs. 76, 77, 78, Pl. 24; the same appendage (first thoracic) from a newly hatched larva is shown in fig. 102, Pl. 25. It has become withdrawn to the level of the surrounding epidermis, and survives as the imaginal disk of the leg, and is recognizable only as a rather deeply staining thickening of the epidermis.

The structure of the mouth-appendages is quite different, for they are not hollow, but consist of a solid mass of long filamentous epidermal cells, the nuclei congregating towards the base of the appendage.

B. The Oenocytes.—These are confined to the abdomen and occur as clusters of cells situated in the fat-body just behind the stigmatic trunks.

They arise, as in other insects, from the epidermis. After the embryo has begun to shorten they become recognizable as small clumps of enlarged cells in the epidermis just behind the stigmata. They separate off, and move into the fat-body, where they enlarge and assume their very distinctive features. The imaginal oenocytes are already sharply distinguishable from the larval by their small size (fig. 115, Pl. 26).

C. The Tentorium.—In the larva this consists of a transverse bar of chitin running through the head-capsule just behind the circum-oesophageal nerve strands (Text-fig. 18), from the base of one maxilla to the other; and of two much thinner bars that pass backwards from the base of the antenna to join the transverse bar some distance from its ends.

It arises late in the third day by the fusion of two pairs of tubular ingrowths of the ectoderm arising (i) at the base of the antenna just anterior to the mandible, (ii) at the hinder angle of the maxilla, between it and the labium. There is no contribution from the hinder angle of the mandible. In this respect its development resembles that of Hydrophilus (Heider), Formica and Chrysomela (Strindberg).

Because the tentorial ingrowths are tubular a transverse

canal is formed across the floor of the head-capsule (fig. 116, Pl. 26); with the chitinization of its inner face the lumen later becomes obliterated.

To the backgrowth from the antenna is attached the enlarging coelomic sac of the antennary segment (section 15), the attachment occurring just anteriorly to the developing sub-oesophageal body. At a later period we find the coelomic sac attached to the transverse bar of the tentorium; it seems, therefore, that the transverse bar is largely derived from the antennary and not maxillary ingrowth.

D. The Tracheal System.—This arises, as usual, from segmental ectodermal ingrowths in the thorax and abdomen. In Calandra these appear before shortening of the embryo has begun; they occur in the three thoracic and first seven abdominal segments (Text-figs. 12, 13, 14).

The mouths of the stigmatic ingrowths are at first very wide, but later become reduced to narrow clefts (cf. fig. 77, Pl. 24; fig. 119, Pl. 26, both representing the prothoracic spiracle).

The stigmatic ingrowths appear in close association with the coelomic sacs, against which they tend to flatten. Lehmann (1926) has already drawn attention to the close relationship between these two structures in Carausius, and discussed its possible implications.

During the period of shortening of the germ-band an approximation of successive stigmata is brought about. By the time the shortening is completed small outgrowths from the flattened ends of successive invaginations fuse and so form the rudiments of the longitudinal trunks, within which a gradually enlarging lumen arises.

The tracheal branches arise late on the third day as hollow outgrowths from the blind inner ends of the stigmatic trunks (fig. 119, Pl. 26). Prominent among these are the vessels to the head, and a large branch that passes backwards to the mid-gut.

During the fourth day all but the first and last stigmata close. The mesothoracic stigma has, throughout, been much reduced (Text-fig. 14).

By the time of emergence all the main larval vessels have developed. Throughout the larval period much elaboration of

the vessels occurs. Their anatomy in the fully developed larva is shown in Text-fig. 9 of our previous paper (Murray and Tiegs, 1935); a drawing from the first instar is given in a paper by Hozawa (1929).

18. THE NERVOUS SYSTEM.

A. Ventral Nerve-cord.—The first indication of the ventral nerve-cord appears at the time the somites are forming. The ectoderm at this period presents a pair of median thickenings (fig. 42, Pl. 22). Beginning at the anterior end and spreading backwards a differentiation now occurs within the thickenings to form an internal layer of pale neuroblasts and an external layer of smaller more deeply staining epidermal cells (dermatoblasts of Wheeler). The segregation is brought about by certain of the cells enlarging, losing their columnar shape and becoming withdrawn from the exterior to form an inner layer (the neuroblasts) while the dermatoblasts between them withdraw to the outside (fig. 43, Pl. 22).

Two continuous lateral cords of neuroblasts thus arise along the length of the embryo, the only indication of segmentation being a succession of lateral intersegmental indentations at their margins. Originally the lateral cords are not more than two to three neuroblasts in width; in later embryos as many as five rows occur in the middle of the segment, reduced to three at the intersegments. The additional cells seem to arise by differentiation out of the ectoderm, and not from already formed neuroblasts, for no proliferation of the latter has been seen.

The origin of nerve-cells from the neuroblasts[1] presents but little variation from that described for other species. In Korotneff's account for Gryllotalpa (1885) and Wheeler's for Xiphidium (1893) the nerve-cells are described as forming by unequal division of the large neuroblasts, the nerve-cells thus arising being small and deeply staining, and forming columns of cells above the parent neuroblasts. In Forficula

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[1] Following His, the term 'neuroblast' is now generally reserved for cells that become directly converted into nerve-cells; it was originally employed for their parent cells and as such has survived in the literature on insect embryology.

several columns occur for each neuroblast (Heymons, 1895 a), in Xiphidium one. In neither species, nor in Eutermes (Strindberg) or Pieris (Eastham, 1930) does subsequent multiplication of the resulting nerve-cells take place; but in Doryphora, according to Wheeler (1893), and in Apis (Nelson, 1915) it occurs.

Early stages of nerve-cell formation in Calandra are seen in fig. 44, Pl. 22; fig. 46, Pl. 23; and fig. 67, Pl. 24; a more advanced stage appears in fig. 47, Pl. 23, the tendency of the nerve-cells to lie in columns being here clearly seen. But in Calandra, unlike most other species investigated, division of the nerve-cells occurs, and is, indeed, quite extensive; mitoses are seen in fig. 47, Pl. 23; figs. 75, 76, Pl. 24; fig. 95, Pl. 25. As it progresses the orderly alignment of the nerve-cells becomes obliterated.

As the two lateral cords enlarge and bulge on to the surface a neural groove develops between them in the position of the old gastral groove (Text-figs. 10–13; figs. 46, 47, Pl. 23; figs. 67, 68, 75, 76, Pl. 24; fig. 89, Pl. 25).

In addition to the two lateral cords the narrow median cord must be distinguished, forming the floor of the neural groove. As in other species its cells (neurogenic cells) are narrow and columnar (fig. 75, Pl. 24) except at the intersegments where rather deeply staining neuroblasts occur flanked at the sides by elongate columnar cells (fig. 93, Pl. 25). The intersegmental position of these median-cord neuroblasts is well shown in the horizontal section (fig. 98, Pl. 25). Unlike the cells of the lateral cords those of the median cord are not covered externally by dermatoblasts, but abut on to the surface of the neural groove.

Cell-division is occasionally observed among the neurogenic cells. Late during the third day they lose their columnar form and, becoming polygonal, assume the appearance of nerve-cells. They retain their original position immediately above the neural groove, and are readily distinguished from the other nerve-cells by their paler cytoplasm and rather larger size. They are now definitely part of the nerve ganglion, forming the roof and part of the lateral walls of the ventral fissure between the unfused right and left halves of the ganglion (fig. 76, Pl. 24).

The incorporation of the median-cord neuroblasts into the developing ganglia is delayed for a time because fusion of right and left halves is slowest at the intersegments. Occasionally they may be observed to divide before this (fig. 97, Pl. 25). Eventually when shortening of the germ-band has become advanced we find them forming a small clump of rather deeply staining cells located on the postero-dorsal wall of each ganglion (fig. 95, Pl. 25). Their incorporation into the ganglia was first observed by Graber (1890); it remained for Wheeler (1893) and Heymons (1895) to note that they became applied only to the hinder wall of the preceding ganglia.

The foregoing observations on the fate of the median cord are substantially in agreement with those of Hatschek (1877), Graber (1890), Heider (1889), and Heymons (1895) who were all able to observe its incorporation into the ganglia; an exception must, however, be made for a few intersegmentally located median-cord cells, which form the neurilemma (q.v.).

Although the median-cord cells lie free at the surface they do not participate in forming the sternal integument, as Hatschek (1877) and Wheeler (1893) maintain; on the contrary the dermatoblasts at the sides of the neural groove occasionally divide and completely close in the median cord from below (figs. 75, 76, Pl. 24). With this the neural groove disappears, the integumental cells becoming withdrawn from the ventral fissure of the nerve-cord which itself then gradually vanishes as the cord thickens (figs. 77, 78, Pl. 24; fig. 115, Pl. 26).

The formation of nerve-fibres begins at about the time the germ-band starts to shorten; it is first seen in the anterior ganglia, thence spreading backwards. The first indication is an elongation of certain nerve-cells, short axons growing out and converging dorsally in each lateral cord (fig. 75, Pl. 24). In the rather more advanced embryo shown in fig. 76, Pl. 24, the axons have elongated and become bifurcate, the 'Punktsubstanz' thus becoming well defined.

From now on the individual ganglia become more sharply demarcated. The median-cord nerve-cells move on to the dorsal surface of the ganglion, covering in the 'Punktsubstanz' above, while the lateral cords fuse almost completely, thereby obliterat-

ing the ventral fissure (figs. 77, 78, Pl. 24). The 'Punktsubstanz' increases in mass, while the axons become of immeasurable fineness. Between successive ganglia lateral connectives begin to appear, and the transverse commissures become defined.

The construction of the ganglia at this period is seen in fig. 97, Pl. 25, representing a horizontal section just under the dorsal surface of the last thoracic ganglion, of an embryo at the stage shown in Text-fig. 13. The median-cord derivative is divided by the anterior and posterior commissures into three parts—the anterior, median, and posterior zones of Graber—the last-named arising only partly from the median-cord neuroblasts, which are recognizable by their large size. To the sides lie the great masses of lateral-cord cells. Nerve axons appear with unusual clearness, and it is possible to see that the transverse commissure develops from both the lateral and median-cord nerve-cells, the latter also contributing to the formation of the longitudinal connectives.

These observations then fully confirm the statement of Heymons on Forficula that 'the entire dorso-median part of the ventral ganglia, inclusive of certain fibres of the transverse commissures, arise from the median cord that originally formed the floor of the neural groove'.

In rather more advanced embryos the nerve-cord becomes separated from the epidermis; lateral nerves are now seen communicating with the myoblasts (fig. 78, Pl. 25; figs. 115, 117, Pl. 26).

During the fourth day the cord enlarges further. This is only partly due to growth of the 'Punktsubstanz', for there is still much evidence of division both of the neuroblasts and the undifferentiated nerve-cells.

The fate of the neuroblasts is hard to determine. In Xiphidium (Wheeler), Forficula (Heymons), and Eutermes (Strindberg) they are said to degenerate. In Calandra they diminish much in size in later divisions, and are therefore hard to distinguish from nerve-cells. Degenerated remains, if they occurred, could scarcely be distinguished from the paracytoids (section 21) which are very common in the advanced nerve-cord (figs. 116, 118, Pl. 26). In some instances degenerated cells have

been encountered at the site of the former neuroblasts, their presence suggesting that the neuroblasts do indeed degenerate (fig. 116, Pl. 26). In Hymenoptera, however, the neuroblasts survive—Carrière and Bürger (1897), Nelson (1915).

The ventral nerve-cord comprises sixteen ganglia, to which must be added a few neuroblasts at the tip of the last abdominal ganglion. The sub-oesophageal ganglion that arises by fusion of the three gnathal ganglia is much enlarged. The thoracic ganglia remain separate. On the fourth day concrescence of the last three abdominal ganglia occurs (Text-fig. 17). The abdominal ganglia thus reduced to eight become further reduced to six after the larva has emerged. In the imago further concrescence occurs, the entire nerve-cord comprising five ganglia, of which the hinder part of the third, together with the fourth and fifth, are to be reckoned as abdominal (Murray and Tiegs, 1935).

The occurrence of a few nerve-cells in the last segment (eleventh) is noteworthy; in L e p i s m a and in some Orthoptera according to Heymons a complete ganglion develops here, as also in C h a l i c o d o m a (Carrière and Bürger).

B. T h e   B r a i n.—Between the neuroblasts of the brain and of the ventral nerve-cord there is no observable difference, either in regard to the time or the manner of their development; the formation of the nerve-cells from the neuroblasts and their subsequent division is also similar (cf. figs. 83, 87, 89, Pl. 25).

The three component ganglia of the brain become defined at the same time as the ventral ganglia. Their position is best seen in ventro-lateral views of entire cleared embryos. The accompanying drawing (Text-fig. 19) is from an embryo which has begun to shorten; the tritocerebral ganglion, continuous behind with the mandibular, lies postero-laterally to the stomodaeum, just in front of the angle of the mandible, and, though sharply defined, is small; the deutocerebral occupies a rather larger area at the base of the antenna; the protocerebral is very large and occupies a great part of the inner surface of the head-lobe.

Histologically the tritocerebral ganglion differs from those behind it only by its median cord; the latter widens out just behind the stomodaeum, is devoid of neuroblasts, and does not take any part in the formation of the mature ganglion.

The early development of the protocerebrum is best examined in longitudinal horizontal section. Fig. 92, Pl. 25, is from an embryo at about the stage of Text-fig. 19. The three component lobes of the protocerebrum are recognizable; posteriorly is the first lobe (optic ganglion), still part of the epidermis, and distinguished by its rather large cells, though neuroblasts are absent; while in front of this, indistinctly demarcated from one another by an ingrowing ridge of dermatoblasts, lie two masses
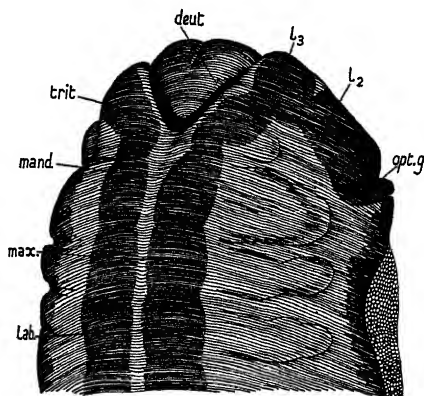
TEXT-FIG. 19.

Anterior end of embryo at about stage shown in Text-fig. 12; drawn to show nerve ganglia (shaded). *deut.*, deutocerebral ganglion; l_2, l_3, second and third lobes of protocerebral ganglion; *lab.*, labial ganglion; *mand.*, mandibular ganglion; *max.*, maxillary ganglion; *opt.g.*, invaginating optic ganglion; *trit.*, tritocerebral ganglion.

of neuroblasts with their progeny of nerve-cells, separation of these two lobes being less evident than in Orthoptera and related forms (Viallanes, Wheeler, Heymons, Strindberg).

Separation of the head-ganglia from the epidermis does not occur till the end of the third day, the ganglia having now become very massive. The deutocerebrum has now merged into the protocerebrum; the tritocerebrum, however, though connected with the deutocerebrum and mandibular ganglion, retains its individuality (fig. 117, Pl. 26).

The 'Punktsubstanz' has now also begun to appear in the

various ganglia, thereby further enlarging and consolidating the brain. In the protocerebrum two such masses arise, namely in the second and third lobe, but soon merge into one; although they form on the internal surface of the ganglia, they later become completely enclosed by nerve-cells (cf. figs. 117, 118, Pl. 26). The interganglionic connectives now also develop, the circum-oesophageal strands becoming prominent (fig. 118, Pl. 26). The transverse commissures require special comment, as their development has been the subject of much discussion.

The sub-oesophageal commissure arises from the tritocerebrum. In Forficula, according to Heymons (1895 a), it develops from median-cord cells associated with that ganglion. This is confirmed by Strindberg (1913) for Eutermes, and by Carrière and Bürger (1897) for Chalicodoma; Nelson (1915) remained uncertain about the point in Apis, while in Pieris, according to Eastham (1930), it is derived not from the median-cord, but from the 'median inner cells of the tritocerebral ganglia'. Paterson (1935) in Corynodes derives it from the ganglion itself. In Calandra the median cord does not participate. This is clearly shown in fig. 117, Pl. 26, representing a frontal section of the head (transversely cut embryo), the section passing along the median cord. The latter is seen passing above on to the stomodaeum, and is quite distinct from the tritocerebral ganglia, from which axons are developing to form the commissure. The fully formed commissure is seen in fig. 118, Pl. 26.

There is a similar difference of opinion for the supra-oesophageal commissure. In Forficula Heymons derives it from the median epidermis, and this is confirmed by Eastham for Pieris and by Paterson for Corynodes. Viallanes (1891, Mantis), Wheeler (1893, Xiphidium), and Strindberg (1913, Eutermes) derive it from the ganglion cells of the protocerebrum and deutocerebrum. In Calandra the epidermis plays no part in its formation, the commissure arising from the protocerebrum, and probably also from the deutocerebrum. An early stage in its formation is seen in fig. 117, Pl. 26; the mature commissure is shown in fig. 118, Pl. 26. In Calandra a median depression of the epidermis, similar to that figured by

other authors, occurs; but it lies just anteriorly to the commissure and is not associated with it; on it are inserted some of the dorsal oesophageal dilator muscles. Theoretically the point is of interest, because Heymons (1901) homologizes the median nerve-cells with the annulate archicerebrum.

The first lobe of the protocerebrum (optic ganglion) is, as already said, devoid of neuroblasts. On the third day the ganglion 'Anlage' begins to invaginate (fig. 92, Pl. 25), the invagination being readily visible in entire embryos as a slightly crescentic surface cleft (Text-fig. 19). Such a cleft has been figured by Patten (1888) for Acilius and by Wheeler (1893) for Xiphidium, and is described also by Viallanes and Heymons.

The fate of the invaginated cells is not known with certainty. It should be observed that in the species hitherto described it is not actually the optic ganglion that is invaginated, but rather a ridge of ectodermal cells ('intraganglionic thickening' of Wheeler). In Calandra, however, it is the ganglion 'Anlage' itself that invaginates, and this makes the cleft easier to follow in later embryos. After separation from the epidermis the ganglion, with cavity still visible despite cell proliferation, is found forming the postero-ventro-lateral part of the protocerebrum (fig. 116, Pl. 26). Heymons suspected that the invaginated cells degenerated. For Calandra this is not the case; the cavity of the invaginated mass becomes reduced to an almost imperceptible cleft, its outer (thick) wall being the optic lobe of the brain, while its inner wall forms the immediately adjacent part of the protocerebrum.

During the fourth day the enlargement of the great head muscles pushes the brain backwards some distance into the thorax. This is attended by elongation of the circumoesophageal connectives, which curve backwards over the transverse bar of the tentorium (Text-fig. 18).

C. The Sympathetic (Stomatogastric) System.—This comprises only three ganglia, pharyngeal ganglia being absent. The frontal ganglion (Text-fig. 18) is relatively large and from it nerves pass to the labrum, oesophageal dilator muscles, and to the oesophagus. The 'recurrent nerve' is short,

the hypocerebral ganglion being an elongate inconspicuous swelling on it (fig. 101, Pl. 25). The ventricular ganglion (Text-fig. 18) is also comparatively small; nerves pass from it on to the adjacent wall of the gut.

There are two connexions with the brain: (i) by a pair of short incurving branches from the cerebral (i.e. fused antennal, labro-frontal and ocellar) nerves, which join the frontal ganglion; (ii) short connectives between the hypocerebral ganglion and the deutocerebrum (in our previous paper the connexion was stated erroneously to be with the tritocerebrum).

In its development the stomatogastric system does not present any novel features. During the third day, in embryos in which shortening has begun, the dorsal wall of the stomodaeum loses its regular epithelial character, its cells enlarging, becoming pale, and assuming an appearance very like neuroblasts (fig. 89, Pl. 25). Unlike those of the brain and nerve-cord, however, they are not teloblasts, but divide by equal division.

The ganglia arise as the usual three median invaginations (fig. 100, Pl. 25), which after separating from the stomodaeum fuse into a continuous cord. Although the invaginations are of about equal size the first (frontal) soon outstrips the others, while the second (hypocerebral) remains small (fig. 101, Pl. 25).

The stomatogastric system is seen in transverse section in figs. 116, 117, 118, Pl. 26.

D. 'Neurilemma'.—This membrane invests the cord, brain, and sympathetic system. Although its flattened nuclei are readily seen, the membrane itself easily escapes detection, hence the difficulty of observing its development.

Korotneff (1885) in Gryllotalpa derived it from amoeboid mesoderm cells, but for most authors it is ectodermal. Wheeler (1893) believed that in Xiphidium its origin could be traced from the intraganglionic portion of the median cord, although Hatschek (1877) had already shown that the latter became an integral part of the nerve-ganglia (Bombyx). According to Heymons (1895 *a*) it seems to arise from the ganglia themselves by flattening out of superficial cells 'which during the segregation of the neuroblasts from the dermatogenic layer separate

from the latter' (Forficula). Strindberg (1913) concluded that in Eutermes they arose from superficial cells of the ganglia, i.e. from the progeny of the neuroblasts, and this is supported by Eastham (1930 a) for Pieris.

In Calandra the neurilemma of the brain and sympathetic seems to be derived from the superficial cells of these organs. But in the ventral nerve-cord it has quite a different origin, being formed from the intersegmental portion of the median cord; in a recent paper Paterson (1935) advocates the same for Corynodes.

The cells concerned are certain long columnar cells already described (section 18 A) as flanking the intersegmental neuroblasts (fig. 93, Pl. 25). These cells become associated, late on the third day with the dermatoblasts lining the neural groove (fig. 94, Pl. 25). When these dermatoblasts now become withdrawn to the level of the sternal integument they remain associated by long filaments with the median-cord cells, the lateral spreading of the dermatoblasts causing thereby the formation of delicate partitions between successive ganglia. Fig. 99, Pl. 25, shows the partition at the thoracico-abdominal intersegment; it is from a transversely cut embryo, a fragment of the right and left hinder wall of the last thoracic ganglion being included in the section. Though best developed in the thorax, the partitions occur in all the segments. Comparable structures have been observed by Wheeler (1893) in Doryphora and Xiphidium and by Heymons (1895 a) in Forficula. In these species they form, in the thorax, apophyses for attachment of leg muscles, while in the abdomen they are said soon to disappear. But in Calandra they form the neurilemma.

In sagittal section they appear as in fig. 95, Pl. 25 (abdominal 3, 4, and 5); the preparation is from paraffin-embedded material, the shrinkage of the ganglia from the epidermis serving to accentuate the connexion between the latter and the partitions. Fig. 96, Pl. 25, is from a later embryo in which shortening is completed (abdominal 3 and 4). It is evident that the intersegmental partition, now closely investing the ganglion, has become the neurilemma, and is still connected below with the

sternal integument. This connexion survives throughout the embryonic period, but becomes less evident after hatching. Whether the neurilemmal cells that invest the lateral walls of the ganglia are derived by the spreading out of these cells, or whether they arise locally from the ganglia, present methods seem quite unable to decide.

The neurilemma of the brain also shows, in places, connexions with the adjacent epidermis, recalling, in this respect, Patten's account for Acilius (1888). When later the brain becomes pressed back into the thorax, these disappear.

19. THE MUSCULAR SYSTEM.

A description of the muscles referred to in the following account is given in our previous paper (Murray and Tiegs, 1935).

A. Muscles of Thorax and Abdomen.—These develop from two sources (i) the external walls of the somites, (ii) the subsomitic mesoderm. From the former arise, as described above (section 10), the lateral plate myoblasts— segmentally disposed masses of cells adjacent to the terga of the body-wall, i.e. dorsal to the spiracles. The subsomitic mesoderm is sternal in position and comprises (*a*) segmentally disposed clumps of cells, lying dorso-lateral to the nerve-cord, to the sides of the epineural sinus; (*b*) masses of cells in the lateral body-wall, ventral to the lateral plate myoblasts; (*c*) myoblasts of the appendages (thorax only) (figs. 76, 77, Pl. 24).

As the body-walls spread upwards over the sides of the egg, the lateral plate myoblasts grow dorsally, separating meanwhile into three masses, of which two are longitudinally disposed, and are the 'Anlagen' of the median-dorsal and dorso-lateral bands of muscle-fibres, while the third, external to these, becomes greatly elongated dorso-ventrally, and is the 'Anlage' of the transverse muscle-bands (fig. 78, Pl. 24; fig. 115, Pl. 26). The myoblasts themselves now become disposed into columns of elongate cells, the terminal cells being inserted on to the adjacent epidermis, usually at the intersegments. In the case of the transverse muscles enlargement of the epidermis between the points of insertion draws out the columns of myoblasts to their definitive length.

The ventral muscle-band develops from the most lateral portion of the subsomitic mesoderm; from the latter arise also, it seems, the first and second oblique muscle-bands, as well as the most ventral part of the transverse muscles, the tergal part of which is derived from the lateral plate myoblasts.

The strongly developed third system of oblique muscles, i.e. most internal, arises from the clumps of subsomitic mesoderm cells lying dorso-lateral to the nerve-cord. A few of the most anterior cells of these masses are in direct contact with a ridge of epidermis that projects inwards intersegmentally to the side of the nerve-cord, while other cells at the hinder ends of these masses, bending outwards, become associated with the epidermis of the lateral body-wall one segment behind. With the spreading of the lateral body-wall over the sides of the egg, late on the third day, the epidermis between these two regions of attachment becomes much enlarged, with the result that the clumps of myoblasts become drawn out into long obliquely running columns of cells (fig. 115, Pl. 26). Thus arises the innermost system of oblique muscles.

In the prothorax occur certain muscles specially adapted for movement of the head. The two head depressors develop from the lateral plate myoblasts of the prothorax (fig. 119, Pl. 26), while the levator capitis is formed from the clumps of myoblasts nearest the nerve-cord.

The myoblasts that occupy the cavities of the thoracic appendages remain dormant till metamorphosis. In the larva they form clumps of cells associated with the imaginal disks of the legs (fig. 102, Pl. 25); from them arises, in the imago, the intrinsic musculature of the legs.

B. Muscles of Head.—The larval antennae are devoid of muscles, a clump of associated myoblasts, derived from the antennary mesoderm, remaining dormant till metamorphosis, when they form the flexor and extensor muscles of the antennae.

From each of the gnathal segments a pair of muscles—flexor and extensor—develop in association with each appendage. (In our previous paper we did not describe muscles in association with the labium; they have been detected in the present material and comprise very minute muscle-fibres, 2 to 3 in number).

In the mandibular and maxillary segments, as already recorded, coelomic sacs are absent and the mesoderm is unsegmented. It occupies the cavities of the appendages and spreads a little outwards on the body-wall. With the beginning of shortening of the germ-band this mesoderm becomes much enlarged, partly by addition of cells from the cavities of the appendages.

The tendon of the great flexor muscle of the mandible arises shortly after this. It develops as a large hollow ingrowth of the ectoderm at the hinder corner of the mandible. This ingrowth comes into association with the rapidly enlarging adjacent mass of mesoderm cells (myoblasts) which now arrange themselves into radiating columns of cells from which the muscle-fibres are formed (Text-fig. 18; fig. 105, Pl. 25; fig. 116, Pl. 26). Chitinization of the tendon occurs late on the fourth day. The much smaller extensor muscle of the mandible arises in the same way, anteriorly to the flexor.

The muscles of the maxilla take their origin from the cross-bar of the tentorium. The association is established by the maxillary component of the tentorium (section 17 C) drawing the myoblasts with it along its path of invagination. The minute labial muscles come from the subsomitic mesoderm.

20. THE GONADS.

The origin of the sex-cells at the hinder pole of the blastoderm has been described in section 4.

With the formation of the germ-band, the sex-cells cease to appear at the surface, becoming overgrown by the germ-band as it bends upwards over the hinder pole of the egg. The elongation of the germ-band carries them by about the middle of the second day to the anterior pole of the egg, where they form a conspicuous clump of rather pale cells just below the surface. Stages in this migration are shown in Text-fig. 6.

From this position at the hinder end of the germ-band the mass moves forwards again, in the developing abdomen, as the proctodaeum elongates. Early stages of this are shown in figs. 60 and 61, Pl. 23; by the end of the second day the mass has moved forwards almost to the eighth segment (fig. 64, Pl. 23).

At about this period the germ-cells are coming into close relationship with the adjacent coelomic sacs, the masses thereby dividing into right and left halves. An early stage of this is shown in the serial sections given in fig. 67, Pl. 24; it will be observed that the division proceeds from before backwards.

The inferior portion of the splanchnic walls of the more posterior abdominal coelomic sacs consists of small, rather scattered cells, without any regular epithelial alinement. From these arises the investing sheath of the gonad. As the germ-cells come into relation with the coelomic sacs, these cells become indented by them into the coelomic cavities, which thus become obliterated (fig. 67 H, fig. 68, Pl. 24). The investing sheath which the germ-cells thus acquire consists at first of scattered cells only, though in later embryos these become consolidated into a continuous membrane (cf. fig. 75, Pl. 24; and fig. 104, Pl. 25, both taken at the level of the seventh abdominal segment; also fig. 103, Pl. 25).

This penetration of the sex-cells into the coelomic sacs occurs in the middle of the third day, just before the beginning of shortening of the embryo, at a time when the cavities of the sacs have already become confluent, and when differentiation of the walls is beginning. From the ninth, eighth, and seventh sacs, where the penetration occurs, they migrate forwards as a compact cord of cells, about three or four segments in length, till they reach, at the end of the third day, the third abdominal segment.

By the time the gonads reach that level the fat-body has become well defined. The gonads now become spherical masses, and lie amongst the fat-cells dorsally in the haemocoele, whither they have been carried by the upward spread of the body-walls over the sides of the egg. There is no connexion with the developing heart as in Orthopteran embryos.

During the fourth day the gonads move back to about the seventh abdominal segment (Text-fig. 17).

The sexual ducts arise late on the fourth day from the mesodermal cells that ensheath the gonad. These cells have, by now, considerably increased in number, and are conspicuous on the inferior surface of the gonad. Shortly before the larva emerges

these cells grow downwards as a pair of solid stalks to the base of the ninth segment, where they impinge on the epidermis.

Shortly after emergence of the embryo the gonad becomes completely encased by fat-body.

Except for the presence of mycetocytes in the ovary, there is no perceptible difference between the latter and the testis.

21. Appendix. The Paracytoids.

Reference must now be made, in this concluding section, to a peculiar cytological phenomenon—paracytoid formation—which is displayed by cells of the embryonic tissues at various phases of the insect's development.

In our previous paper on the metamorphosis of Calandra this phenomenon was described under the name of 'chromatic globule extrusion', and its similarity shown to the process observed by Poyarkoff twenty-five years earlier in the metamorphosis of Galerucella. It consists in the extrusion of minute pieces of chromatin from the nucleus into the cytoplasm, where they apparently swell, cohere, and after receiving an investing film of cytoplasm, become cast out from the cells into the blood. It occurs not only for cells which, like the epidermal and tracheal cells, have contributed to the formation of the larva, but also for cells which have remained embryonic during larval life, e.g. the imaginal myoblasts. We now find the same process occurring during the development of the embryo within the egg; examples are clearly seen in the following illustrations: fig. 34, Pl. 22; figs. 51–60, Pl. 23.

The formation of these globules in the embryo is best examined in the ectodermal cells, at a period prior to formation of coelomic sacs. A cell with well-developed globule is shown in fig. 80, Pl. 23. The globule is large and rounded and is contained within a vacuole. Sometimes it stains uniformly deep with haematoxylin; more often it exhibits one or more deeply chromatic clumps lodged within a pale spherical matrix. In depth of staining the chromatic clumps compare with the chromatin of dividing nuclei, and much exceed that of the resting nuclei. They give the specific chromatin reaction with Feulgen's reagents, while the surrounding pale matrix appears green if 'light green'

counterstain is applied. Whether it is cytoplasm, or extruded plastin material, needs investigation.

Although, then, largely of nuclear origin, the chromatic globules do not arise in their mature form from the nucleus, for the nuclei present, at most, only small chromatic inclusions (nucleoli?) and even these are usually not seen. If it is legitimate to reconstruct their manner of formation from their appearance in other cells, then it would seem that comparatively small particles are extruded into the cytoplasm from the nucleus, a film of cytoplasm (?) condensing round them, while at the same time the chromatic particles swell and eventually cohere into larger drops. The occurrence of swelling must be inferred from the fact that the globules may, at times, exceed a normal nucleus in size.

Eventually the globules are extruded from the cells into the intercellular spaces, whence they find their way mostly into the yolk. Here they are to be seen lying in small clusters, particularly at the posterior and anterior ends of the germ-band (figs. 51, 52, 54, 60, Pl. 23). Some of them have much enlarged, either by further swelling or by fusing with other globules.

In the extensive literature on insect embryology reference is frequently made to a peculiar phenomenon of 'Paracyten' formation, consisting in the extrusion of modified cells (Paracyten) from the embryonic tissues into the yolk. They were first described by Heymons (1895 a) in Orthoptera and Dermaptera, and (1901) in Scolopendra, and have since been reported by other authors—Friedrichs (1906), Schwartze (1899), Strindberg (1913), Wiesmann (1926); while the frequent references to passage of degenerate cells into the yolk probably relates to the same. In appearance the Paracyten are like the 'chromatic globules' above described; they are, however, in all cases, transformed cells, and not of intracellular origin. Such Paracyten are occasionally seen also in Calandra (fig. 82, Pl. 24), but are of infrequent occurrence.

Friedrichs has, however, described in the development of Donacia a process which seems to be identical with that observed in Calandra. From the true Paracyten he distinguishes these bodies of intracellular origin as 'Paracytoids'.

They arise from the germ-cells, ectoderm, and mesoderm, and are cast out into the yolk. Except that they are stated to arise also from yolk-nuclei in D o n a c i a they resemble the chromatic globules of C a l a n d r a so closely as to justify the adoption of Friedrichs' designation for the latter.

As to their significance nothing is known. They are not an expression of cell-degeneration, for the cells containing them are otherwise normal in appearance, and, indeed, are not infrequently seen in mitosis (fig. 81, Pl. 24). Nor do they seem to be concerned with digestion of yolk, as suggested by Friedrichs, for similar globules are shed by the serosa and amnion into the extra-embryonic fluids (fig. 64, Pl. 23). Nor do the time and place of their occurrence yield any clue; they appear about the middle of the second day in the ectoderm, and especially, but by no means exclusively, at the anterior tip of the germ-band; also in the sex-cells and embryonic membranes. In some parts, such as the head lobes, they may, indeed, be surprisingly numerous. Later they appear, though usually only sparsely, in the mesoderm and the mid-gut 'Anlage' (cf. figs. 51–5, Pl. 23). During the third day they are seen within various organs which are arising at the time (fat-body, corpora allata, tracheae, splanchnic muscle, and particularly the nervous system). Their degree of prevalence in any tissue is not, however, a real measure of the frequency of their formation, for their apparent preponderance in the nerve-cord and brain of later embryos is probably due to the difficulty of eliminating them out of such massive organs into the blood. Within the brain they occur even after the larva has hatched.

There is no evidence for their association with any visible histological differentiation of tissues; while Poyarkoff's suggestion—that they are the expression of a dedifferentiation of specialized cells preceding redifferentiation into those of the imago—is excluded on the ground of their occurrence in the developing egg.

Are the paracytoids perhaps a device for maintaining a nucleo-cytoplasmic ratio in rapidly multiplying cells?

SUMMARY.

1. In the maturation of the egg, although post-reduction appears to occur, there is actually pre-reduction, but much obscured owing to a separation of precociously split chromosomes late in the first meiotic anaphase. A temporary separation of conjugated chromosomes also precedes the first meiotic division.

2. Cleavage (non-synchronized) follows rapidly upon fusion of male and female pro-nuclei. The cleavage-cells spread through the yolk, apparently by their own activity. A cleavage pattern, though not directly observable, is to be inferred on theoretical grounds.

3. The cleavage-cells become drawn into the periplasm by a centrifugal flow of the cytoplasm. Upon entering the periplasm, or just before this, the nuclei divide. Early cleavages in the blastoderm are therefore synchronized. Later the synchronization disappears, though other remarkable forms of co-ordination have been encountered. As the blastoderm matures, the cells, now much diminished in size, develop first lateral and then inner cell-walls, the latter within the secondary periplasm.

4. The yolk-cells are derived from a small number of cells that do not enter the periplasm. They divide apparently solely by mitosis. The blastoderm does not contribute appreciably to their number.

5. The germ-cells are part of the blastoderm, and protrude prominently at the hinder end. Later they become withdrawn level with the blastoderm. They early become infected with bacteria from a large bacterial mass in the adjacent yolk. The mycetocytes of the ovary arise at this time by migration of adjacent blastoderm-cells into the bacterial mass.

6. The germ-band arises, as usual, by a dorsal thinning and a ventral and lateral thickening of the blastoderm. Beginning at the anterior end the median and two lateral plates become demarcated, the latter then invaginating with formation of a temporary gastral groove. The invaginated cells form the inner layer, which is entirely mesodermal.

7. The germ-band, meanwhile, grows over the hinder pole of the egg and extends to the anterior end, carrying the germ-cells with it. Associated with this is a peculiar method of amnion-formation due to deep invagination of the germ-band into the yolk; on the ventral surface of the egg the amnion arises by downgrowth of folds along the margin of the germ-band.

8. Segmentation of the germ-band proceeds from before backwards, without the formation of macrosegments. A vanished twelfth segment is inferred for the abdomen. The appendages develop approximately in order from before backwards. The labrum appears later. In the abdomen there are no appendages. Shortening of the germ-band occurs on the third day, and thereafter the larval form is gradually assumed, the lateral body-wall growing upwards over the yolk. The formation of the head is described in detail. The thoracic appendages merge into the body-wall in the advanced embryo, and form, then, the imaginal disks of the legs.

9. Amnion and serosa do not rupture, but form a permanent enclosure for the embryo.

10. The stomodaeum marks the anterior limit of the invaginated inner layer, the pre-oral mesoderm arising by the spreading forward of cells from behind the stomodaeum. The post-oral mesoderm differentiates into a median unsegmented sheet of cells and two lateral rows of somites, extending from the labial to the tenth abdominal segment; elsewhere the mesoderm remains unsegmented. With the exception of the tenth abdominal, these somites expand into coelomic sacs. A coelomic sac forms later in the antennary segment also.

11. On the third day, the cavities of the coelomic sacs having become confluent, differentiation proceeds: the splanchnic wall forms the splanchnic muscle of the gut; the inferior wall becomes the fat-body; the dorso-lateral wall forms the heart tissue, while the lateral wall becomes resolved into the lateral plate myoblasts, from which much of the somatic musculature develops.

12. The remaining somatic muscles are derived from the sub-somitic mesoderm, into which is incorporated most of the median unsegmented mesoderm.

13. With the withdrawal of the median mesoderm the epi-neural sinus is formed between the yolk and the nerve-cord; by its enlargement the haemocoele is developed.

14. The stomodaeum and proctodaeum arise as simple in-growths of the outer layer, the latter occurring at the posterior limit of the germ-band.

15. The mid-gut has a bipolar origin. It forms from the blind ends of the stomodaeum and proctodaeum, quite inde-pendently of the inner layer. The mid-gut 'Anlagen' grow towards one another and meet in the first or second abdominal segment. They eventually wholly enclose the yolk.

16. The difficulty of reconciling such facts with the germ-layer theory is discussed.

17. The malpighian tubes arise from the proctodaeum, the first pair much preceding the other two in time of development.

18. The remarkable adaptation of the development of the intestine to the bacterial symbiont is described.

19. The sub-oesophageal bodies arise from the mesoderm just anterior to the mandible, then become part of the mid-gut wall, but later lose association with it. They survive even into the imago.

20. The corpora allata do not arise from the ectoderm, but from the inferior wall of the antennary coelomic sac.

21. The dorsal blood-vessel and associated tissues arise from the dorso-lateral walls of the two rows of coelomic sacs, which meet in the mid-line above the gut and enclose a tube. The cephalic aorta is derived from the antennary coelomic sacs. The blood-cells are formed exclusively from a narrow median ridge of mesodermal cells above the nerve-cord.

22. The tracheal system arises from ten pairs of stigmatic invaginations from the prothoracic to the seventh abdominal segment. At their blind ends these expand to form the two main longitudinal vessels; from their blind ends the branching tracheae to the tissues also grow out. In the advanced embryo all but the first and last stigmatic openings close.

23. The nerve-cord arises early as a pair of ventral thickenings of the outer layer (lateral cords), within which the cells differ-entiate into dermatoblasts and neuroblasts. The latter are

teloblasts, and bud off a succession of nerve-cells, which them-
selves further divide. The median cord, between the two
lateral cords, contributes to the formation of the ganglia; its
intersegmental neuroblasts attach themselves to the postero-
median wall of the ganglia, while the intra-segmental (neuro-
genic) cells form the roof of the completed ganglia, their axons
contributing to the formation of the transverse commissures
and longitudinal connectives. Sixteen ganglia form in the
nerve-cord, the first three uniting into the sub-oesophageal
ganglion, while the last three abdominal also fuse.

24. The three component ganglia of the brain are clearly
defined in the embryo. In the protocerebral ganglion the usual
three lobes are seen. The optic ganglion does not contain
neuroblasts and arises by invagination from the surface. Unlike
the ventral nerve-cord, there are no median cord components
in the brain.

25. The stomatogastric system arises as three invaginations
from the roof of the stomodaeum.

26. The neurilemma of the ventral nerve-cord is derived
from certain intersegmental median-cord cells. In the brain and
sympathetic it is derived from the ganglia themselves.

27. On the third day the germ-cells move forward in the
abdomen, enter the hinder coelomic sacs and press forward as
a solid cord of cells to the third abdominal segment. They form
here a spherical mass of cells and are now encased in fat-body.
The genital ducts arise from splanchnic mesoderm cells en-
sheathing the gonads.

28. A peculiar cytological phenomenon—paracytoid forma-
tion—appears at the time of germ-band formation and in later
phases of development. It is indistinguishable from the 'chro-
matic globule extrusion' already described from the meta-
morphosis of Calandra. Its significance is unknown.

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EXPLANATION OF PLATES 21 TO 26.

REFERENCE LETTERS.

a., amnion; *a.c.s.*, antennary coelomic sac; *adv.*, adventitia; *al.*, alary muscle; *ant.*, antenna; *bac.*, bacterial mass; *b.l.*, blood-cell lamella; *br.*, brain; *c.*, cardioblast; *c.a.*, corpus allatum; *c.o.c.*, circum-oesophageal connective; *cr.*, crop; *c.s.*, coelomic sac; *d.a.c.*, dorsal amniotic cavity; *db.*, dermatoblast; *deu.g.*, deutocerebral ganglion; *dev.my.*, developing mycetocytes; *ep.*, epidermis; *ep.s.*, epineural sinus; *f.b.*, fat-body; *f.b.a.*, 'Anlage' of fat-body; *f.c.*, flanking cells of median cord; *g.c.*, germ-cells; *h.*, heart; *haem.*, haemocoele; *i.l.*, inner layer; *l.*, leg; l_2 and l_3, second and third lobes of protocerebral ganglion; *lb.*, labium; *l.b.s.*, lateral blood sinus; *l.m.p.*, lateral myoblast plate; *l.p.*, lateral plate; *lr.*, labrum; *mal.*, malpighian tube; *m.c.*, median cord; *m.c.n.*, median cord neuroblast; *m.d.c.$_1$* and *m.d.c.$_2$*, first and second depressor capitis muscles; *m.dors.lat.*, dorso-lateral muscle; *mes.*, mesoderm; *m.g.*, mid-gut cells; *m.lev.cap.*, levator capitis muscle; *m.med.dors.*, median dorsal muscle; *mn.*, mandible; *mn.g.*, mandibular ganglion; *m.obl.$_3$*, third oblique muscle; *m.p.*, median plate; *m.trans.*, transverse muscle; *m.vent.*, ventral muscle; *mx.*, maxilla; *my.*, myceto-cytes; *myb.*, myoblasts; *n.*, nerve; *nb.*, neuroblast; *n.c.*, nerve-cell; *nl.*, neurilemma; *o.d.*, oesophageal dilator muscle; *oen.*, oenocytes; *opt.g.*, optic ganglion; *p$_2$*, secondary ·periplasm; *pa.*, paracardial cells; *p.b.w.*, provisional body-wall; *pe.*, pericardial (adventitial) cells; *p.o.m.*, pre-oral mesoderm; *p.o.m.g.*, pre-oral mid-gut cells; *pr.*, proctodaeum; *pr.g.*, protocerebral ganglion; *pr.m.m.*, premandibular (post-oral) mesoderm; *r.c.*, 'replacing' cells; *s.*, serosa; *sal.*, salivary gland; *sb.o.c.*, sub-oesophageal commissure; *s.g.*, sheath cells of gonad; *si.*, sinus; *s.o.b.*, sub-oesophageal body; *s.o.c.*, supra-oesophageal commissure; *som.*, somite; *sp.*, spiracle; *sp.h.*, sperm head; *spl.m.*, splanchnic mesoderm; *s.s.m.*, sub-somitic mesoderm; *st.*, stomodaeum; *sy.*, sympathetic (stomatogastric) nerves; *t.*, tentorium; *tr.*, tracheal vessel; *tr.g.*, tritocerebral ganglion; *v.a.*, vascular 'Anlage'; *v.a.c.*, ventral amniotic cavity; *v.n.c.*, ventral nerve-cord; *y.*, yolk; *y.m.*, limiting membrane of yolk.

All drawings made with aid of a camera lucida.

PLATE 21.

Fig. 1.—Diplotene stage of first meiotic division; perinuclear sheath intact; only a small rectangular area of 'perinuclear substance' drawn. From egg in last ovarian chamber. ×1,400.

Fig. ·2.—Exconjugant chromosomes in contracted condition, with a tendency of some to undergo precocious division; perinuclear sheath intact; all that remains of 'perinuclear substance' has been drawn. From egg in last ovarian chamber. ×1,600.

Fig. 3.—Nucleus in periplasm; 12 chromosomes present, some partially divided; sheath and perinuclear substance disappeared. Vaginal egg. ×1,600.

Fig. 4.—Equatorial plate of first meiotic division; note indication of tetrad formation in some of the chromosome pairs. Newly laid egg. ×1,400.

Fig. 5.—First meiotic anaphase; many of the chromosomes are already partially split. Ten minutes after laying. ×1,600.

Fig. 6.—Late anaphase, polar body beginning to protrude; division of some of the chromosomes has now occurred, about 9 chromosomes (chromatids) occurring at the polar body end, 8 at the oocyte end. ×1,600.

Fig. 7.—Late anaphase, with 9 chromosomes (chromatids) at polar body end, 9 or 10 at oocyte end. ×1,600.

Fig. 8.—Very late anaphase, the oocyte chromosomes being seen in polar view; 11 chromosomes present, of which one is dividing. Photograph. ×2,200.

Fig. 9.—First polar body formed; nuclear membrane invests oocyte nucleus; in polar body 10 chromosomes can be counted, though some of these may be double; in oocyte nucleus there are 12 chromosomes. Twenty-five minutes after laying. ×1,600.

Fig. 10.—Early stage in pairing of oocyte chromosomes. Rather less than 1 hour after laying. ×1,600.

Fig. 11.—Advanced stage of same. ×1,600.

Fig. 12.—Equatorial plate of second meiotic division. Fifty-five minutes after laying. ×1,600.

Fig. 13.—Second meiotic anaphase, with 6 chromosomes passing to each pole. ×1,600.

Fig. 14.—Late anaphase, nuclear membrane disappearing. First polar body still protruding. The drawing has been made sufficiently deep into the egg to include a sperm-head lodged in an accumulation of cytoplasm. Sixty-five minutes after laying. ×900.

Fig. 15.—Telophase; nuclear membrane disappeared. ×1,600.

Fig. 16.—Migration of female pro-nucleus ($♀n$) towards male pro-nucleus ($♂n$); first polar body ($p.b.1$) is withdrawn into periplasm; second polar body ($p.b.2$) is a 'resting nucleus'. Reconstructed from two sections. ×400.

Fig. 17.—Longitudinal section of egg just prior to fertilization; male and female pro-nuclei are lodged in the prominent mass of cytoplasm towards anterior end of egg; polar-plasm protruding into yolk. Eighty minutes after laying. ×120.

Fig. 18.—From the same egg, showing male and female pro-nuclei just prior to fertilization. ×800.

Fig. 19.—Fertilization, with intermingling of chromosomes. ×1,600.

Fig. 20.—Second polar body becoming resolved again into its chromosomes; from same egg as figs. 17 and 18. ×1,600.

Fig. 21.—Degenerating polar bodies; chromosomes of second polar body distinguishable by their greater length. From an egg at 14-cell stage. ×1,600.

Fig. 22.—Nucleus of cleavage-cell in prophase. From egg at 6-cell stage. ×1,400.

PLATE 22.

Fig. 23.—Transverse section of egg, about 1 hour after laying, showing sperm lodged in crescentic accumulation of cytoplasm. × 280. Photograph by Dr. E. S. J. King.

Fig. 24.—First cleavage; from a longitudinally cut egg. The strip of egg drawn includes its whole width; note periplasm at either side. × 410.

Fig. 25.—Two cleavage-cells from egg at 6-cell stage, to show connexion of adjacent cells with one another and with periplasm. Both cells in mitosis. × 520.

Fig. 26.—Division of cleavage-cells immediately before entering periplasm; no uniform orientation of spindles. Note the rich intravitelline mesh of cytoplasm external to line of nuclei, in contrast to its poverty internal to the line. × 520.

Fig. 27.—Cleavage-cells entering periplasm; note well formed asters. Nuclei in prophase of mitosis. × 600.

Fig. 28.—Fragment of newly formed blastoderm; nuclei in prophase of division; asters visible. × 550.

Fig. 29.—Fragment of young transversely cut blastoderm; synchronized mitoses. Blastoderm mitoses with tangential spindles; one nucleus, not actually within the blastoderm, showing radial spindle; 2 yolk-cells dividing. × 550.

Fig. 30.—Posterior end of longitudinally cut blastoderm, with protruding mass of germ-cells. The large dark object in yolk anterior to germ-cells is the bacterial mass. Some organisms are seen penetrating the adjacent secondary periplasm; others are already lodged within or among germ-cells. Within the blastoderm lateral cell-walls have formed, but internal walls are yet absent. × 600.

Fig. 31.—Fragment of longitudinally cut blastoderm with well-developed secondary periplasm, within which the inner cell-wall is developing. × 450.

Fig. 32.—Posterior end of longitudinally cut blastoderm (female), showing entrance of blastoderm-cells into the bacterial mass (mycetocyte formation). From the secondary periplasm the inner cell-walls of blastoderm and outer limiting membrane of yolk have developed. × 600.

Fig. 33.—Male, at about same stage of development. × 600.

Fig. 34.—Transverse section through hinder end of germ-band of an embryo at about the stage shown in Text-fig. 9. Section passes through genital rudiment; note associated mycetocytes. Number of paracytoids present. × 440.

Fig. 35.—Very early stage in differentiation of blastoderm into serosa and germ-band; transverse section. × 440.

Fig. 36.—Transverse section through anterior end of embryo drawn in Text-fig. 4; differentiation of germ-band into lateral and median plates. × 440.

Fig. 37.—Transverse section through rather older embryo, with median plate invaginating, and development, on one side only, of amnion. × 440.

Fig. 38.—Transverse section through posterior end of embryo shown in Text-fig. 5. The invagination of inner layer is still in progress on the dorsal surface, but is practically complete ventrally. Gastral groove distinct. ×440.

Fig. 39.—Transverse section of embryo at stage of development shown in Text-fig. 6 B. Along the ventral surface the inner layer is completely separated from lateral plates. Dorsally the development is less advanced. ×440.

Fig. 40.—Transverse section from near hinder end of an embryo at about the stage shown in Text-fig. 7. Embryonic membranes more advanced. Inner layer spreading outwards. ×440.

Fig. 41.—Sagittal section of an earlier germ-band than the foregoing. The section passes along the future sixth to ninth abdominal segments and shows heaping up of inner-layer cells to produce a rudimentary segmentation. ×440.

Fig. 42.—Transverse section through abdomen, showing lateral spreading of inner layer (early phase). ×500.

Fig. 43.—Later stage, showing developing somites. Differentiation of neuroblasts in the outer layer. ×500.

Fig. 44.—Portion of transverse section of embryo at stage shown in Text-fig. 9. The section passes through the mandible (to right), and shows the mesodermal cells forming a lining to its cavity. There are three small nerve-cells present, first products of fission of the neuroblasts. ×500.

PLATE 23.

Fig. 45.—Longitudinal section through three abdominal segments (abd. 3–5) with advanced development of somites. Note separation of successive somites. ×330.

Fig. 46.—Transverse section through abdominal segment with well-developed coelomic sac. Precocious formation of epineural sinus, due to withdrawal of mesoderm to the sides. In the developing nerve-cord neuroblasts and a few nerve-cells are seen. ×330.

Fig. 47.—Transverse section through labial segment, showing mid-gut 'Anlage'. Transformation of coelomic sac has not yet begun. 'Blood-cell lamella' present but not conspicuous; subsomitic mesoderm enters cavity of appendage. Nerve-cord better defined; nerve-cells more numerous, one in mitosis. ×420.

Fig. 48.—Longitudinal section through first two thoracic segments, from an early 3–day embryo, to show communication between successive coelomic sacs. ×420.

Fig. 49.—Sagittal section of anterior end of very young germ-band, showing thickening of outer layer preliminary to formation of stomodaeum. Note heaping of inner-layer cells immediately behind it. ×330.

Fig. 50.—Similar section from a rather later embryo; stomodaeum beginning to invaginate. ×330.

Fig. 51.—Similar section showing the stomodaeum immediately prior to mid-gut formation. It is hemispherical but its walls have preserved their epithelial character. Formation of paracytoids very intense; some have entered yolk. × 330.

Fig. 52.—Similar section. Beginning of mid-gut formation. Stomodaeum now cylindrical, the hinder wall losing its epithelial character. Several cells near its tip seem about to move out as the mid-gut 'Anlage'. × 330.

Fig. 53.—Similar section. The hind wall of stomodaeum has now completely lost its epithelial character and has given origin to a large mass of cells which is growing back, as the mid-gut 'Anlage', over the inner-layer cells. × 330.

Figs. 54 and 55.—Two similar sections showing mid-gut 'Anlage' growing back over the inner layer. The cells have assumed the character distinctive of mid-gut cells. In fig. 55 paracytoid formation is very intense within the mid-gut 'Anlage'. Note pre-oral mesoderm in both figures. Fig. 54—× 330; fig. 55—× 510.

Fig. 56.—Similar section (from an embryo with open coelomic sacs). Mid-gut 'Anlage' now completely separated from stomodaeum, which has regained its regular epithelial character. × 330.

Fig. 57.—Stomodaeum in transverse section; i.e. from a horizontally cut embryo. The section is from an early embryo, in which the mid-gut 'Anlage' is just forming from hind wall of stomodaeum. Note disorganization of epithelium in latter. The mid-gut 'Anlage' is spreading laterally and is also growing forward round the stomodaeum into the head. × 510.

Fig. 58.—Transverse section through part of head-lobe of early germ-band, in which the mid-gut 'Anlage' is forming; the section passes through the hinder wall of the stomodaeum. The mid-gut cells are sharply demarcated from the stomodaeum, and in such a section give no evidence of their origin from the latter. The mid-gut cells are growing forwards round the stomodaeum, over the inner-layer cells. × 300.

Fig. 59.—Complete transverse section through same embryo as shown in last figure (the section is one to the rear, i.e. just behind stomodaeum). Dorsal flexure included in section. Mid-gut 'Anlage' appears as transversely extended mass of cells above inner layer (mesoderm). On one side an antenna is included in section, with mesoderm growing into it. In the dorsal flexure (abdomen) a somite is cut in the right half, while to the left the section passes intersegmentally. Several paracytoids in ectoderm. × 300.

Fig. 60.—Sagittal section through hinder end of young female germ-band, showing proctodaeum in development. Note mycetocytes in genital rudiment. A developing malpighian tube is included in section. × 430.

Fig. 61.—Similar section from rather later embryo; male (mycetocytes absent). Ninth and tenth abdominal somites included in section, the tenth not demarcated from mesoderm of eleventh segment. × 330.

Fig. 62.—Transverse section through hinder end of abdomen, at about same stage of development as in last figure. Proctodaeum a dorso-ventrally compressed cleft with thin roof but thick floor, from which the first pair of malpighian tubes is arising. Note the large unpaired mass of mesoderm in the eleventh segment. × 330.

Fig. 63.—Sagittal section through hinder end of abdomen, immediately prior to formation of mid-gut from it; dorsal wall of proctodaeum much thickened, but proctodaeum still closed at its inner end. × 400.

Fig. 64.—Sagittal section of anterior end of egg immediately prior to shortening of embryo; anterior head-segments ventral, five terminal abdominal segments dorsal. Mid-gut 'Anlage' now separated from stomodaeum, which is elongate and is liberating from its tip a long trail of cells that migrate into the yolk. The proctodaeum has begun to open on to the yolk, while the mid-gut 'Anlage', arising from its tip, is growing over the germ-cells almost to the level of the eighth coelomic sac. Note paracytoids in the extra-embryonic fluids. × 330.

Fig. 65.—Terminal sagittal section along abdomen of an embryo a little more advanced than that shown in Text-fig. 12. Proctodaeum now tubular and widely open; mid-gut 'Anlage' more strongly developed. The mesoderm of eleventh segment has begun to ensheath the proctodaeum. × 330.

Fig. 66.—Transverse section through hinder end of abdomen, from embryo at about stage shown in Text-fig. 13. Proctodaeum tubular; three pairs of malpighian tubes present; mesoderm invests proctodaeum and forms also serous membrane for malpighian tubes; haemocoele developing. × 330.

PLATE 24.

Fig. 67 A–H.—Eight successive sections of a series cut through the proctodaeum at beginning of formation of mid-gut from it. In all the drawings the (true) ventral surface is below, although in the dorsally flexed embryo its position is still inverted. The series illustrates: (i) opening out of roof of proctodaeum at its internal end (B, C, D), and the origin of the two bands of mid-gut cells from the floor of the proctodaeum (E, F, G, H); (ii) division of the genital rudiment and its association with the coelomic sacs. The origin of the first pair of malpighian tubes is seen in B; in A the base of the second pair is just recognizable, while the first is cut transversely now. In A note association of mesoderm cells with malpighian tubes. The level of the series is indicated by the ninth coelomic sac in C and D, the eighth in G and H. × 480.

Fig. 68.—Section from a rather more advanced embryo at same level as that shown in fig. 67 G, i.e. through eighth coelomic sac. The mid-gut 'Anlage' is now a continuous sheet of cells between germ-cells and yolk. × 480.

Fig. 69.—Section along junction of oesophagus (crop) and mid-gut from an advanced embryo. Section not quite in sagittal plane and hence includes

part of sub-oesophageal body. Mycetoma has emerged from yolk. Note fine membranous partition between crop and yolk. × 640.

Fig. 70.—Sagittal section along the mycetoma, as it is emerging from the yolk. × 600.

Fig. 71. Section through fragment of hinder portion of mid-gut of late 4-day embryo, showing intestinal caecum arising as solid outgrowth from its wall. × 800.

Fig. 72.—Fragment of malpighian tube from very late embryo, seen in surface view; lumen dimly visible. Note large and small nuclei of larval and imaginal cells respectively. A thin serous membrane invests the tube. × 800.

Fig. 73.—Section along junction of mid- and hind-gut, with partition still intact. × 800.

Fig. 74.—Section through first abdominal coelomic sac at the beginning of differentiation; adjacent ectoderm, mesoderm, and mid-gut 'Anlage' also drawn. The inferior wall of the sac is losing its epithelial character, and will form the fat-body. × 660.

Fig. 75.—Part of a section through embryo just before beginning of shortening, to show formation of provisional body-wall. The section passes below through the first thoracic segment, above through seventh abdominal (note seventh spiracle and coelomic sac). × 470.

Fig. 76.—Part of a section through first thoracic segment of a rather more advanced embryo. The coelomic sac has become differentiated into lateral myoblast plate, splanchnic muscle and vascular 'Anlagen'. The fat-body is differentiating. Blood-cells are forming from the 'blood-cell lamella', and the epineural sinus is just beginning to appear. × 500.

Fig. 77.—Transverse section through first thoracic segment of an embryo at about the stage shown in Text-fig. 13. The body-wall is extending upwards and thereby encroaching on the provisional body-wall. From the enlarging mid-gut 'Anlage' cells are migrating into the yolk and are transforming into mycetocytes. The epineural sinus has enlarged and contains blood-cells. The mesodermal elements have also enlarged and the myoblast groups have become more sharply demarcated. The prothoracic spiracle is conspicuous. In the nerve-cord the median-cord cells have roofed in the 'Punktsubstanz'. × 500.

Fig. 78.—Transverse section through first thoracic segment of an embryo which has almost completed shortening. A further differentiation of myoblasts into muscle 'Anlagen' has occurred. From the lateral myoblast plate have arisen the median-dorsal, dorso-lateral, and transverse muscle 'Anlagen'; from the sub-somitic mesoderm the ventral and third oblique 'Anlagen' are defined, but the others are not yet delimited. The ventral muscle 'Anlage' has become innervated from the cord. The lateral blood-sinus has formed, and merges below into the epineural sinus. × 310.

Fig. 79.—Longitudinal section of head-end of embryo a little less advanced than that shown in Text-fig. 13. The section is well out of the sagittal plane, structures ventro-lateral to the stomodaeum being included with

the latter in the same section. Mycetocytes are present in numbers at base of stomodaeum. The sub-oesophageal body is now part of the mid-gut wall. The anterior and posterior components of the tentorium have fused, and a fragment of the lower wall of the antennary coelomic sac is seen connected with it. The mass of cells above the stomodaeum is pre-oral mesoderm, not sympathetic 'Anlage'; the post-oral (premandibular) mesoderm associated with it is much less conspicuous. × 400.

Fig. 80.—Paracytoid formation. Three ectodermal cells, one containing an exceptionally large paracytoid. From an early germ-band. × 1,000.

Fig. 81.—Two dividing mesoderm cells from a 2½-day embryo, showing paracytoids. × 1,000.

Fig. 82.—Paracyten formation by direct conversion of entire cell; ectoderm of early germ-band. × 1,000.

PLATE 25.

Fig. 83.—Part of a transverse section of embryo just prior to shortening; section passes below through stomodaeal opening, above through hinder end of proctodaeum. Provisional body-wall developing, and coming into association above with dorsal amnion. In developing brain several layers of nerve-cells have arisen from the neuroblasts; tritocerebral ganglion also present. The section is just behind base of antenna and includes left antennary coelomic sac. × 450.

Fig. 84.—From the same embryo, one section behind, to show sub-oesophageal body. Hinder end of left antennary coelomic sac present. × 450.

Fig. 85.—Fragment of horizontal section along embryo at about stage of Text-fig. 12. The section passes along the ventral body-wall; the orientation will be understood from the position of the antenna and mandible on the right. The large mass of cells on the left is a fragment of the ventral wall of the stomodaeum. Note the premandibular mesoderm behind it. The sub-oesophageal body is seen at the anterior angle of the mandible; some mid-gut cells appear adjacent to the yolk. × 450.

Fig. 86.—Fragment of a transverse section at same level as in fig. 84, i.e. through the tritocerebral ganglion (to left of drawing). The embryo is rather less advanced (note that the antenna has not moved as far forwards as in figs. 83 and 84). The right antennary coelomic sac is seen at base of antenna. The cells of the sub-oesophageal body are already histologically differentiated, but are still part of mesoderm. × 450.

Fig. 87.—Longitudinal section along anterior end of embryo at beginning of shortening to show position of antennary coelomic sac. The section is in the sagittal plane, but to side of mid-line, and passes through base of antenna (left) and mandible (right). × 470.

Fig. 88.—Portion of longitudinal section of same embryo as in fig. 79. The antennary coelomic sac has become pushed back by enlargement of the brain; its connexion with the tentorium is plainly seen. Note that the ventral wall of the coelomic sac is beginning to thicken; it will become the corpus allatum. × 400.

Fig. 89.—Transverse section through anterior tip of an embryo at be-
ginning of shortening. The section passes through the hinder wall of the
antenna. The large mass of cells to left of the antenna is the tentorial
ingrowth cut along its path of invagination; part of the tentorium is also
cut transversely. The antennary coelomic sac is beginning to move back-
ward; its floor is thickening to form the corpus allatum. With the retreat
of the yolk from the head, the haemocoele has begun to form. The stomo-
daeum has acquired an investing sheath. The large ganglion beneath it is
the mandibular. ×470.

Fig. 90.—Lower end of antennary coelomic sac, with developing corpus
allatum. From rather older embryo. ×1,000.

Fig. 91.—The same; the body has acquired an investing sheath, ten-
torium not drawn. ×1,000.

Fig. 92.—Section along the protocerebrum, from a horizontally cut em-
bryo (cf. Text-fig. 19). Note absence of neuroblasts in optic ganglion.
×450.

Fig. 93.—Fragment of nerve-cord in transverse section, to show structure
of the median cord at an intersegment. Three median-cord neuroblasts
(one with paracytoid) and two 'flanking-cells' present. From an embryo
a little before shortening, at level of 4–5 abdominal intersegment. ×660.

Fig. 94.—From a rather later embryo, at labial-prothoracic interseg-
ment. The 'flanking-cells' have separated from the median-cord neuro-
blasts and from the lateral cords, but are closely associated with the
dermatoblasts. ×660.

Fig. 95.—Sagittal section of ventral nerve-cord of embryo after shorten-
ing has begun. Fourth abdominal ganglion and parts of fifth and third
shown. Note median-cord neuroblasts now forming median-postero-dorsal
part of ganglion. 'Punktsubstanz' developing. The interganglionic parti-
tions are the developing neurilemma. ×660.

Fig. 96.—The same, at completion of shortening of embryo. Third
abdominal ganglion and part of fourth shown. Neurilemma now closely
invests ganglion. Interganglionic connective has formed. ×660.

Fig. 97.—Horizontal section along roof of second thoracic ganglion of
an embryo at the stage of Text-fig. 13. Anterior and posterior transverse
commissures have formed; also the interganglionic connectives. Median-
cord neuroblasts still recognizable. ×400.

Fig. 98.—Horizontal section along second thoracic segment, together
with fragment of first and third. From embryo at time of shortening.
The section passes along median cord, and shows the intersegmental
position of its neuroblasts. To the sides of the median cord are seen the
lateral cord neuroblasts. ×300.

Fig. 99.—Intersegmental partition between last thoracic and first
abdominal segment, seen in a transversely cut embryo. The cells have
spread laterally as the neural groove became straightened out. To the sides
the diverging hinder walls of last thoracic ganglion. ×400.

Fig. 100.—Sagittal section along stomodaeum, showing sympathetic ganglia arising as three invaginations from its dorsal wall. From left to right: ventricular, hypocerebral, and frontal ganglia. × 450.

Fig. 101.—A similar section from a 4-day embryo; sympathetic separated from stomodaeum; only part of frontal ganglion contained in section; hypocerebral a barely visible swelling. Inner tip of stomodaeum has become largely converted into mycetocytes. × 300.

Fig. 102.—Imaginal disc of first thoracic leg, from newly hatched larva. Fat-body, two muscle-fibres, and a clump of imaginal myoblasts of the leg-bud included in drawing. × 500.

Fig. 103.—Longitudinal section along lateral margin of an embryo rather less advanced than that of Text-fig. 13. From right to left the third to sixth abdominal segments are seen. Adjacent to the yolk is the vascular 'Anlage', still partially segmented; below lie the sex-cells, encased in a sheath. × 330.

Fig. 104.—Transverse section through fragment of seventh abdominal segment of an embryo rather earlier than the foregoing, to show position of sex-cells. × 330.

Fig. 105.—Part of a section through anterior end of embryo after completion of shortening. The section is so directed as to include the tentorium below, the first thoracic segment above. The antennary coelomic sac, now much elongated, is thus included along its whole length. Below, it is connected with the tentorium; above, it has come into relation with the cardioblasts. × 300.

Figs. 106 and 107.—Two stages in development of cephalic aorta, from embryo in fourth day. In the transversely cut embryo the developing aorta is cut longitudinally, for it is bending down over the yolk towards the stomodaeum (shown below). The inner walls of the two coelomic sacs have become continuous each with a row of cardioblasts, which are now apposed; the outer wall is continuous with the adventitia. Fragment of brain shown to side of aorta. × 600.

Figs. 108, 109, and 110.—Three stages in the differentiation of the vascular 'Anlage', taken at the level of the first alary muscle; fig. 108 from embryo at stage of Text-fig. 13, fig. 110 at completion of shortening, fig. 109 intermediate. In fig. 108 is seen, adjacent to the ectoderm, a conspicuous mass of cells derived from the outer wall of the somite; in fig. 109 its differentiation into cardioblast and accessory vascular cells, alary muscle, and somatic myoblasts is seen; in fig. 110 the differentiation has gone farther. × 580.

PLATE 26.

Fig. 111.—Section through mid-dorsal body-wall of advanced embryo at level of last alary muscle. The two rows of cardioblasts have become apposed, and enclose a cleft-like cavity, which at this period communicates through the 'mesentery' with a narrow sinus round the hinder end of the mid-gut. × 600.

Fig. 112.—The same from newly hatched larva. The heart is now tubular with a spacious lumen, and has lost its association with the mid-gut wall. × 600.

Figs. 113 and 114.—Two adjacent sections, from a sagittal series, of the heart of an advanced embryo. Fig. 113 passes along the row of cardioblasts and shows two ostia; fig. 114 is more lateral and shows the adventitia (pericardium) and segmental masses of paracardial cells (nephrocytes). × 320.

Fig. 115.—Transverse section through third thoracic segment of an advanced embryo. The yolk, which contains a mass of mycetocytes is now almost invested by mid-gut wall. The haemocoele is much enlarged. The fat-body has extended further dorsally. A clump of oenocytes is present. The muscles have become more clearly defined, one being innervated from the nerve-cord.

Fig. 116.—Transverse section through head of embryo at completion of shortening. The section passes through the transverse bar of the tentorium. The brain is cut, towards its hind end, through the optic ganglion, in which a cavity is still present. To the right are fibres of the mandibular flexor muscle. The antennary coelomic sac is much elongated. The corpus allatum is no longer connected with the tentorium. In the mandibular ganglion are seen a number of paracytoids. Two neuroblasts to the left of the 'ventral fissure' seem to be degenerating. × 420.

Fig. 117.—Frontal section of head, from a transversely cut embryo at stage of Text-fig. 13. The deutocerebral ganglion has merged into the protocerebral, but the tritocerebral is still distinct. Nerve-axons are developing, the supra-oesophageal commissure being already distinguishable, while the 'Punktsubstanz' of the deutocerebral and tritocerebral ganglia is in course of formation. Note that the latter forms independently of the median cord (here cut along its length as it bends on to the front of the head and merges into the oesophagus). × 350.

Fig. 118.—Similar section from an advanced embryo, showing the fully developed commissures. × 350.

Fig. 119.—Portion of transverse section through prothorax of an embryo at completion of shortening, to show development of certain specialized muscles. × 700.

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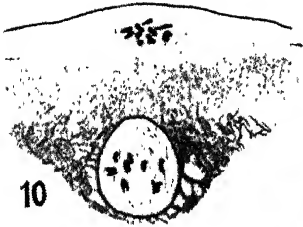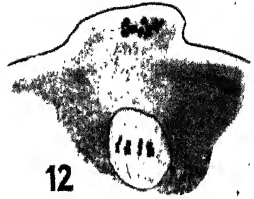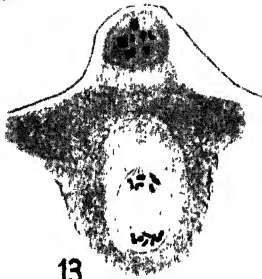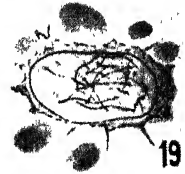
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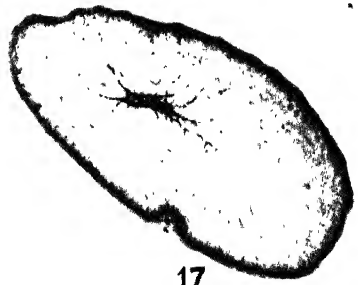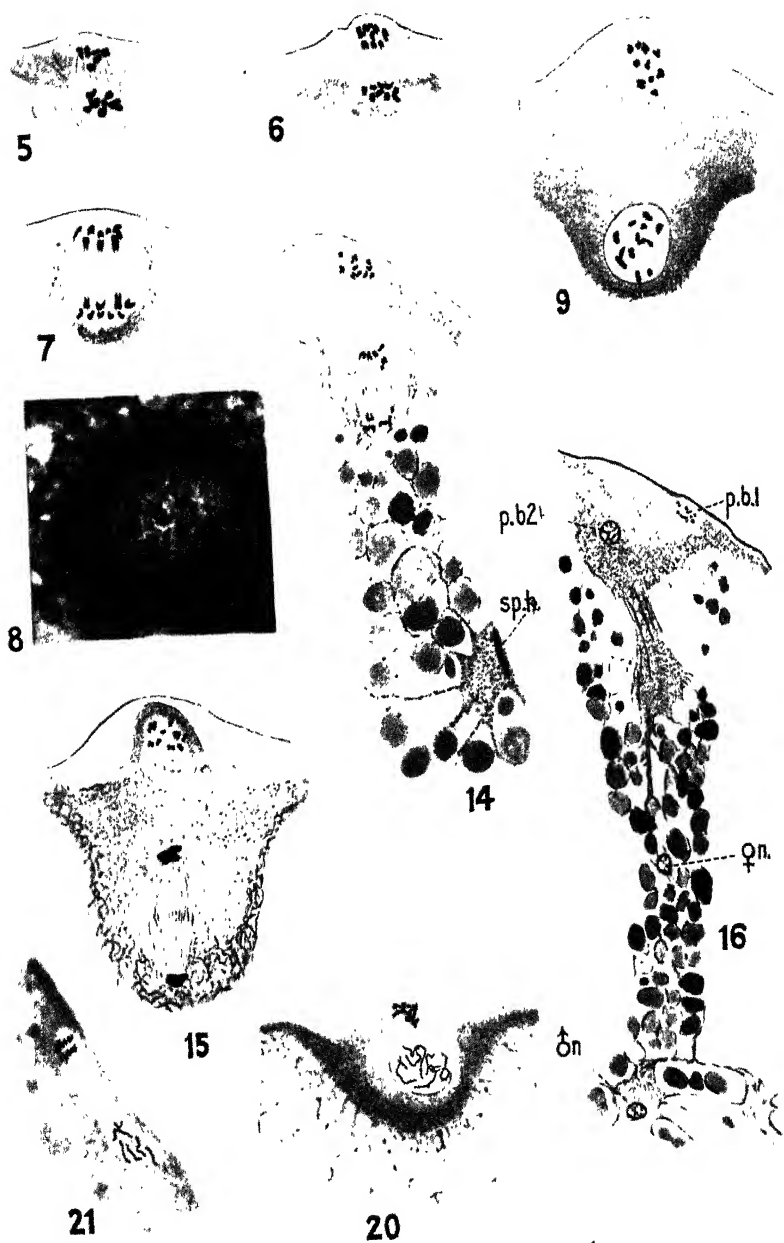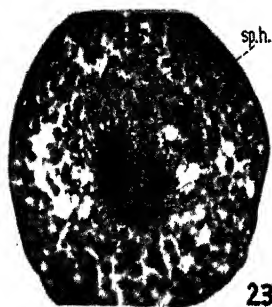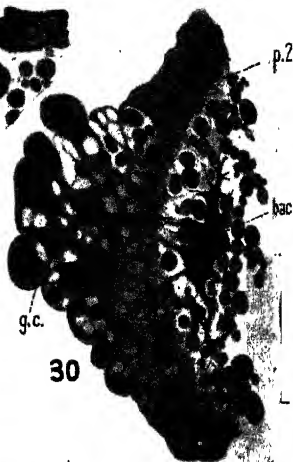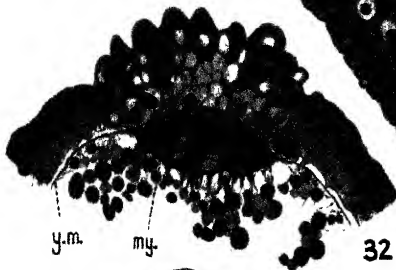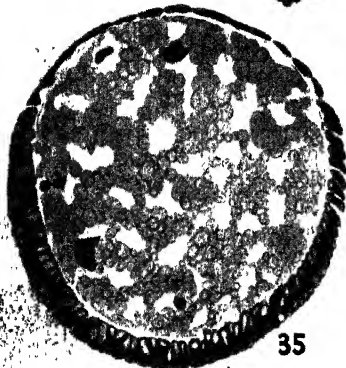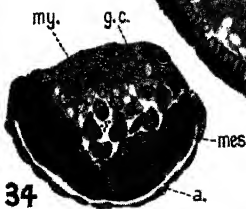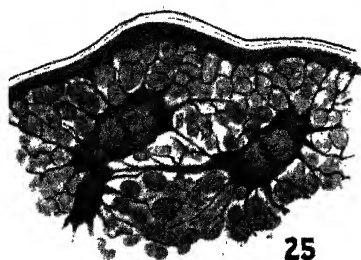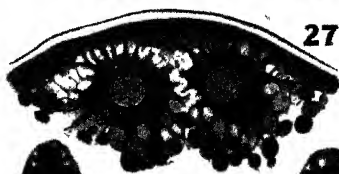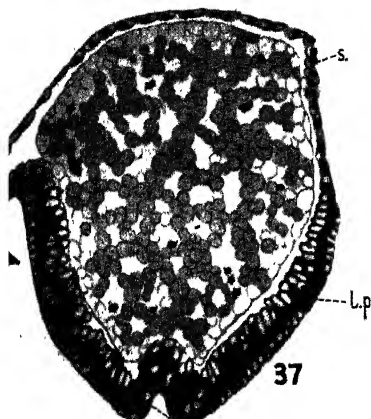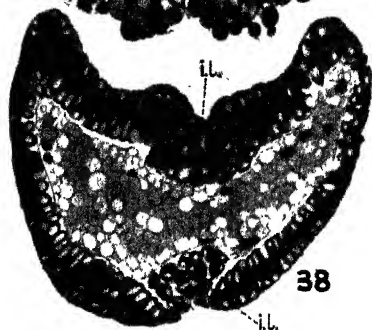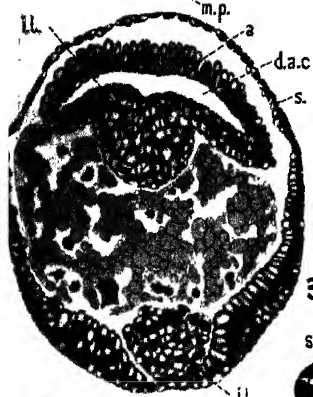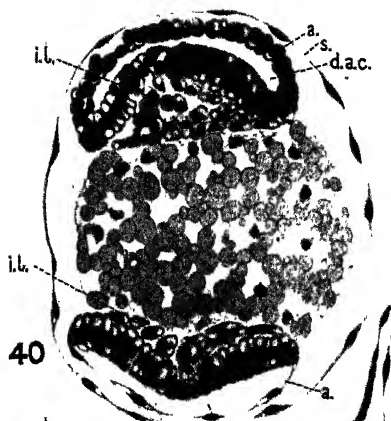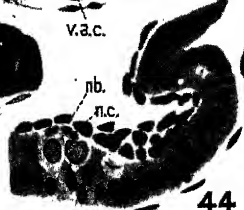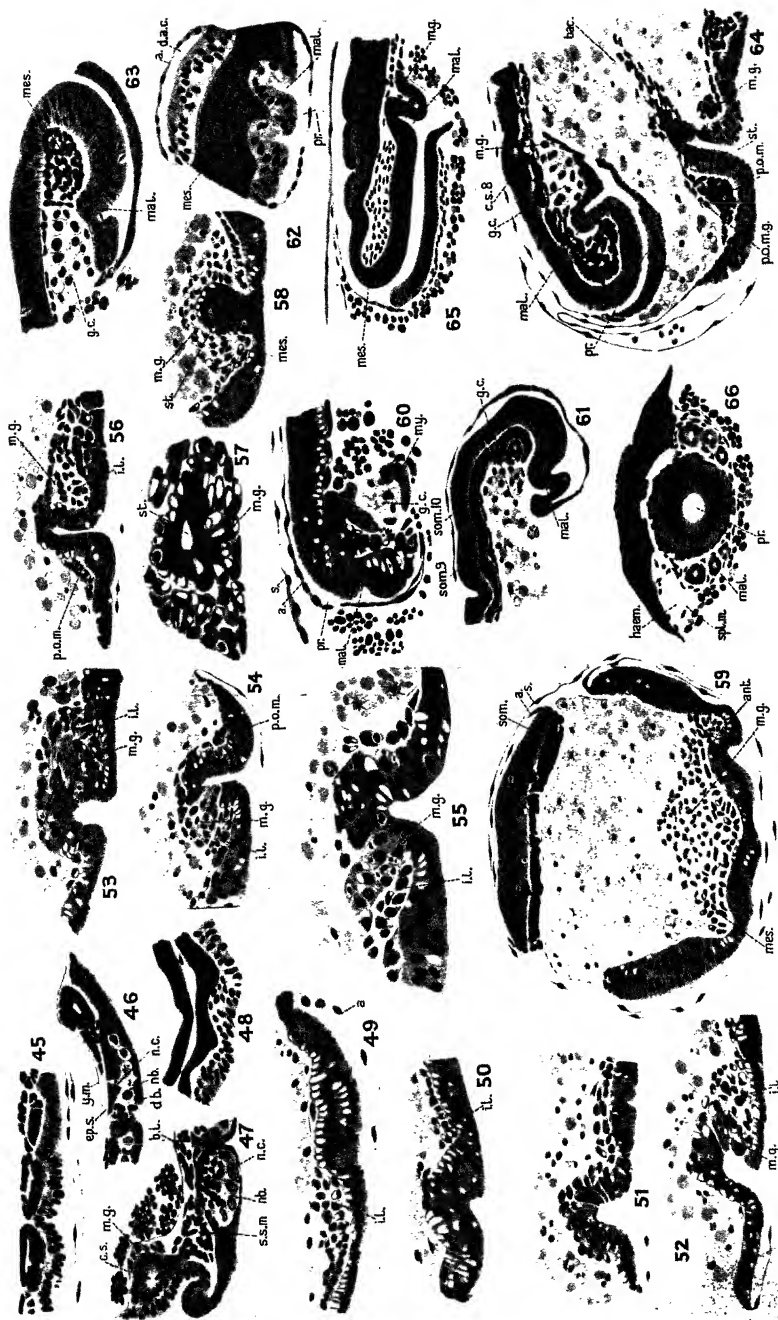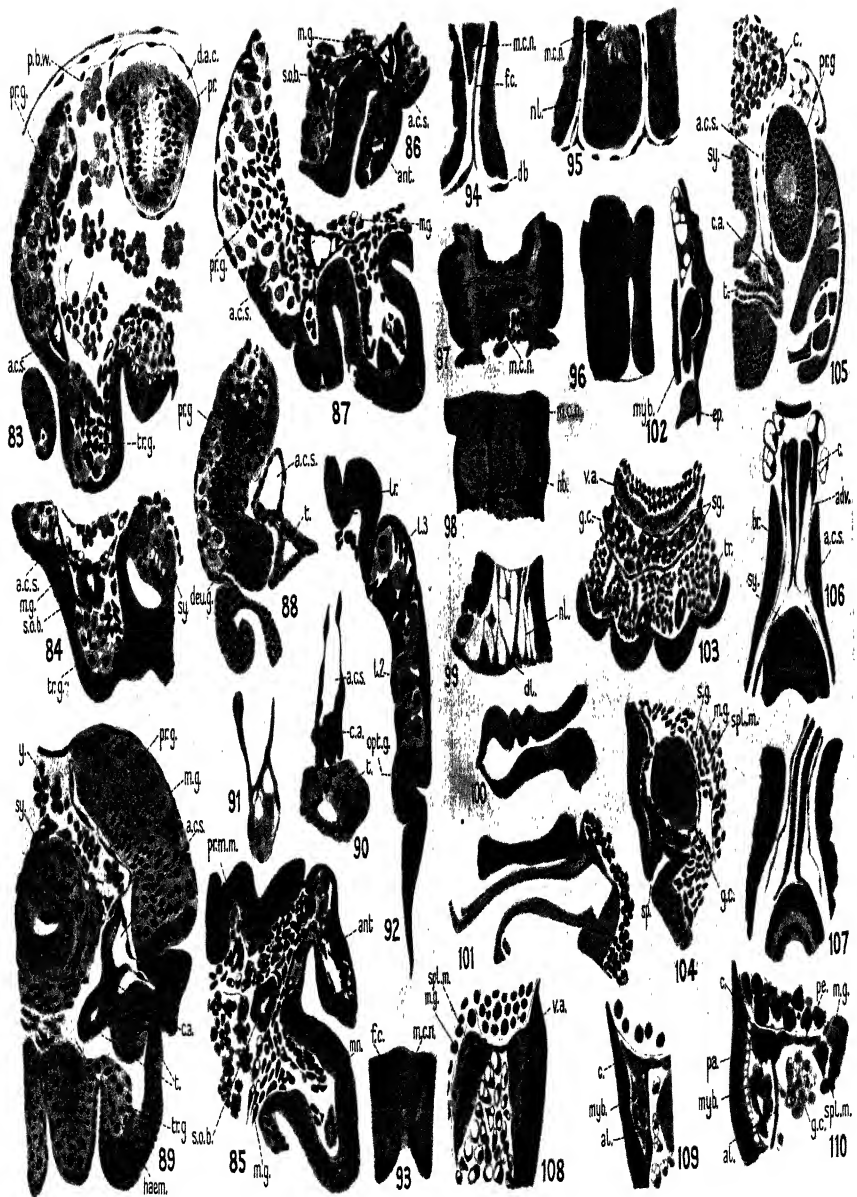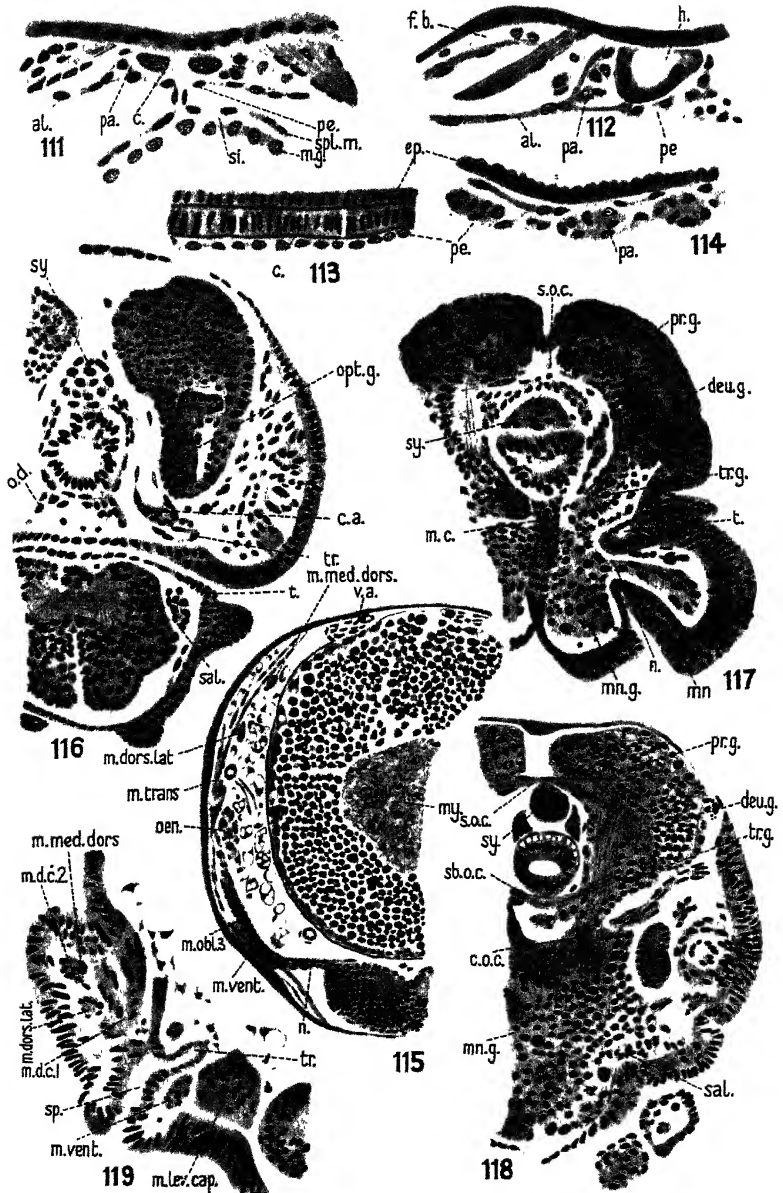
A Quantitative Study of the Changes in the Cytosome of Eggs of the Echinodermata during Early Cleavage.

By

D. Pelluet,

Zoological Laboratory, Dalhousie University, Halifax, N.S., Canada.

With Plate 27 and 2 Text-figures.

1. Introduction.

The early stages in the development of the sea urchin, Arbacia punctulata, occur when the embryos are enclosed in a fertilization membrane through which there is an exchange of sea-water; but no food is taken in, the animal deriving energy for its rapid growth from the reserve food materials stored in the egg cytoplasm. It is well known that these eggs contain fat, yolk, and mitochondria (Morgan, 1927), from which, it may be supposed, that the larva obtains its supply of energy. It was thought that if changes in the quantities of fat and mitochondria occurred, these could be estimated quantitatively by counting the numbers of granules of these substances which appear in material prepared by the usual cytological methods. In addition, eggs preserved in this way and carefully sectioned were measured in order to find out if significant changes in volume occurred during the period of fertilization and cleavage up to the young blastula stage.

2. Methods and Materials.

The eggs of Arbacia were collected at the Marine Biological Laboratory, Wood's Hole, Mass, U.S.A., during July. The ovaries were removed from the sea urchins and the eggs were allowed to shed into finger bowls of sea-water (Just, 1928). After being washed, they were inseminated and the samples were collected at various intervals.
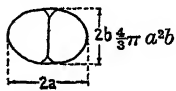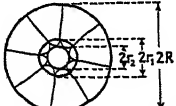
The eggs were collected from several finger bowls, and transferred at once to fixatives which preserve both fat and

mitochondria, viz. Regaud, Flemming without acetic acid, and Champy-Kull (Lee, 1928). The material was imbedded in paraffin after having been dehydrated and cleared in butyl alcohol.

In order to estimate the volume of the larvae at different stages, and to count the granules of fat, as well as the mitochondria, it was necessary to cut sections of these eggs which were 1μ in thickness. This presented considerable difficulties, but finally many slides were produced with uncrushed sections which could be used for measurements.[1] It was not possible to obtain serial sections of single eggs, but in measuring diameters it was comparatively easy to judge which sections had gone through the centre, although this is no doubt a source of error.

The forms assumed by cleaving eggs do not, on the whole, admit of being subjected to comparison with geometrical figures. A mathematician[2] was asked to devise a formula for the two-cell and blastula stages, since these presented the greatest difficulties. Eggs tend to be spherical before and after fertilization, and also in the four-cell stage as may be seen in fig. 1, Pl. 27; thus the

TABLE I.

Age.	Formula.	Volume (μ^3).
Unfertilized	$\frac{4}{3}\pi R^3$	$100,100\,\mu^3$
Fertilized	$\frac{4}{3}\pi R^3$	$86,170\,\mu^3$
two-cell	$\frac{4}{3}\pi a^2 b$	$127,900\,\mu^3$
four-cell	$\frac{4}{3}\pi R^3$	$99,770\,\mu^3$
Blastula	$\frac{4}{3}\pi R^3 - \frac{4}{3}\pi\{\frac{1}{2}(r_1+r_2)\}^3$	$125,132\,\mu^3$

[1] I am indebted to Mr. H. Rifkin, a graduate student, who cut many of the successful sections and repeated most of my fat counts.

[2] My thanks are due to Professor J. G. Adshead, of the Department of Mathematics, Dalhousie University, for suggesting these formulae, after looking at the sections.

ordinary formula for a sphere is used for these stages. Table I gives the formula which is used in each stage and the volume obtained from the measurements made with a micrometer eye-piece which had been previously calibrated.

The errors involved in measuring the diameters or radii were of the order of 2·3 per cent., but other sources of error may account for the fact that the volumes vary markedly, but not in any one direction. The fixing fluids may cause different degrees of shrinking and swelling in the different stages, and changes in volume might be brought about by raising the temperature of the water in which the animals were reared, since the temperature during July of the laboratory in Wood's Hole is about 21° C. or more. In this connexion it is interesting to compare Koehler's (1912) figures for the variation in the volume of Strongylocentrotus larvae produced by changes in temperature which he estimated from whole mounts.

The estimation of the number of fat granules and mito-chondria was carried out by means of a squared disc placed in the eye-piece of the microscope. Text-fig. 1 shows the appear-ance of a section of a four-cell stage and the squares of the disc drawn with a camera lucida at the two magnifications which were used in the counting. The fat-granules were quite distinct in unstained sections, and the counting was carried out with an apochromatic objective (3 mm.) and a compensating ocular (10x). The mitochondria are exceedingly small and can only be counted in perfectly cut and stained sections, using rather brilliant illumination with an oil immersion lens.

The appearance of the fat globules in unstained sections is illustrated in figs. 1, 2, and 3 in Pl. 27.

The fat appears as blackened granules irregularly spaced in the cytoplasm, with a tendency to clump in various parts of the cell. These granules were soluble in turpentine after immersion of the slide for a period of 2 hours or more.

In order to eliminate the personal element in counting the fat granules, my counts were repeated by an assistant[1] to whom my results were unknown. In this way it was found that these independent counts agreed very well, any doubtful cases being

[1] See note 2, on p. 2.

discarded. The percentage errors of all the counts were worked
out, and the results are expressed within the limits of these
errors in Table I (Text-fig. 2).

The counting of the mitochondria was extremely difficult
owing to their small dimensions and the necessity for producing
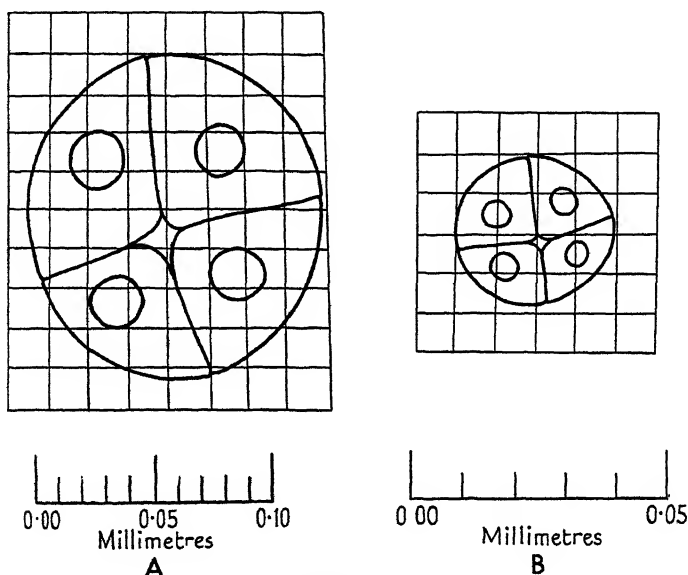
TEXT-FIG. 1.

Text figure 1. Cross section of four-cell stage and squared disc at
the magnification used for (A) counting fat, and (B) counting mito-
chondria,

perfection of staining and illumination. It was not possible to
have independent counts made by a second person, as in the case
of the fat, but all the counts were repeated after an interval of
time had elapsed, which allowed the figures to be forgotten.

The results of the counts for fat and mitochondria and the
errors for each stage are given in Table II. The numbers are
expressed as occurring in a unit volume of the egg, namely,
$100\,\mu^3$, so that the relative numbers of granules can be compared.

These figures are expressed graphically in Text-fig. 2.

The most striking point is the sharp decline in volume, fat,
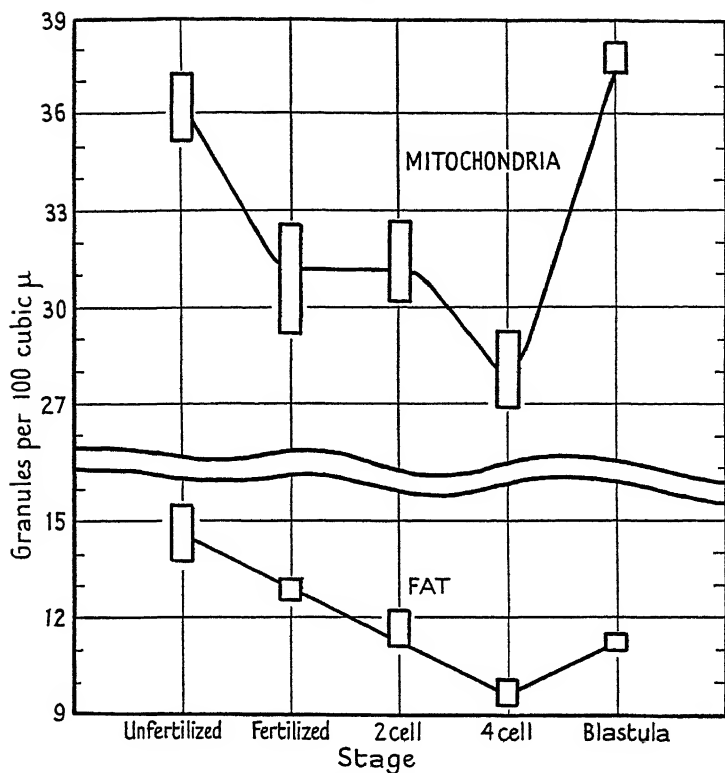
TEXT-FIG 2.

Graph representing the changes in numbers of fat-granules and mitochondria in the early cleavage stages of Arbacia larvae,

TABLE II.

Stage.	Number of Fat-Granules per $100\,\mu^3$.	Per cent. error Fat Counts.	Number of Mito- chondria per $100\,\mu^3$.	Per cent. error for Mito- chondria Counts.
Unfertilized eggs .	13·87–15·53	5·7	35·01–37·39	3·3
Fertilized egg (no cleavage . .	12·69–13·31	2·4	29·07–32·13	5·0
2 cells . . .	11·39–12·61	5·1	30·02–32·38	3·8
4 cells . . .	10·04–09·36	3·5	26·72–29·28	4·6
Blastula . . .	11·03–11·57	2·4	37·25–38·15	1·2

and mitochondria at the four-cell stage, although the fat is decreasing steadily before this, while the mitochondria show a distinct decrease between the unfertilized and fertilized egg with no change until the four-cell stage is reached, when a sharp increase follows. As the intervening stages have not been investigated as yet it is not possible at this time to draw any conclusions.

A similar investigation will shortly be completed on the developing eggs of Asterias, which are markedly different from those of Arbacia, not only in their lack of easily visible fat-globules in unstained preparations but also in the larger size of the mitochondria. Figs. 4 and 5, Pl. 27, show the details of the cytoplasm, containing numerous yolk-droplets and the spherical, evenly distributed mitochondria.

3. Conclusions and Summary.

An estimate of the changes in volume, fat, and mitochondria occurring in the early cleavage stages of Arbacia has been made. The volume fluctuates with each successive cleavage, but it is probable that there is no significant change until after the gastrula stage has been reached.

The number of fat-granules decreases steadily from the unfertilized to the four-cell stage, with a slight increase in the blastula.

The mitochondria show a slight decrease between the unfertilized and fertilized condition and a marked decrease at the four-cell stage, followed by a sharp increase in the blastula.

A preliminary investigation of another Echinoderm, Asterias forbesi, suggests that it differs considerably from that of Arbacia.

It is a pleasure to acknowledge the assistance of Dr. F. R. Hayes in several ways, and especially for checking many of the calculations.

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EXPLANATION OF PLATE 27.

LETTERING.

f., fat-granules; *m.*, mitochondria; *N.*, nucleus; *n.*, nucleolus; *y.*, yolk-granules.

All drawings were made with the camera lucida.

Fig. 1.—Section of A r b a c i a larva, four-cell stage, showing fat-granules. Flemming without acetic fixation. Unstained.

Fig. 2.—Young blastula of A r b a c i a. Champy-Kull. Unstained.

Fig. 3.—Scale of magnification for figs. 1 and 2.

Fig. 4.—Oblique section of four-cell stage (A r b a c i a) Champy-Kull fixation and stain. The left half shows distribution of fat-granules only, other details of the cytoplasm being omitted. On the right-hand side, two areas have been drawn under the squared disc, showing actual numbers of fat and mitochondria.

Fig. 5.—Unfertilized egg of A s t e r i a s f o r b e s i. Regaud fixation, Bensley-Cowdry stain.

Fig. 6.—Same as fig. 5, but with Flemming without acetic fixation, unstained, in which mitochondria appear as greyish refractive granules.

Fig. 7.—Scale for figs. 4, 5, and 6.

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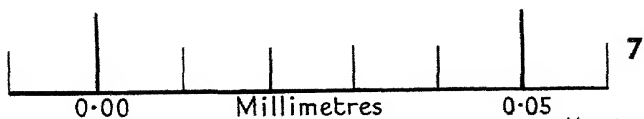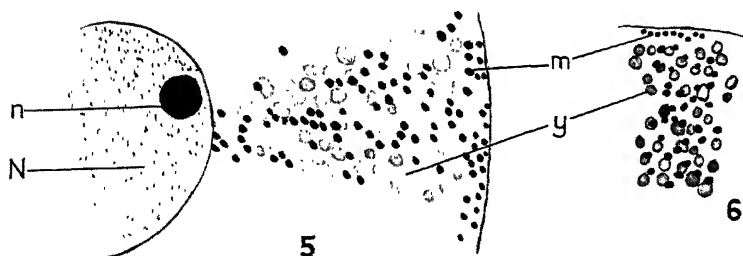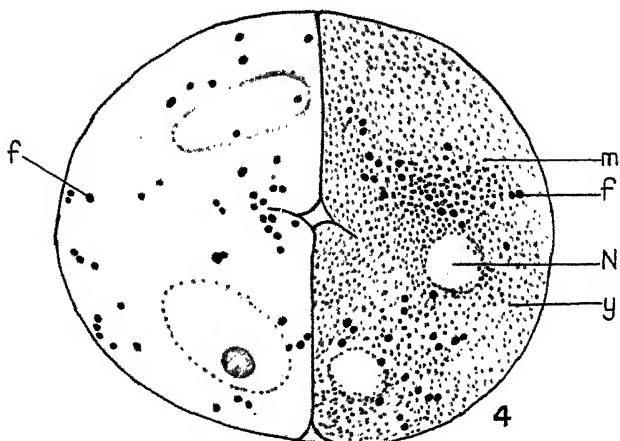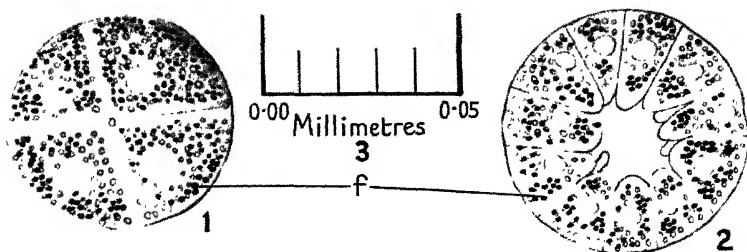
A cytological study of the Gregarine Parasites of Tenebrio molitor, using the Ultra-centrifuge.

By

Margaret I. Daniels, B.A., M.Sc.,

Department of Zoology, Trinity College, Dublin.

With Plate 28 and 2 Text-figures.

INTRODUCTION.

THE ultra-centrifuge, developed by J. W. Beams, has been used for much cytological work on metazoan cells and its importance to cytologists can hardly be over-estimated. It provides a new method of attacking problems that formerly relied alone on fixing, staining, and morphological evidence for their solution. As yet, little work has been done using this method on the Protozoa. In America, King and Beams (1934) have investigated the cytoplasm of P a r a m o e c i u m by this means, and in this department protozoan structure has been examined thus. Patten and Beams (1936) have described the effects of ultra-centrifuging some free-living flagellates, and R. Brown has also worked on P a r a m o e c i u m (communicated).

The present paper deals with Sporozoa, in which a number of different cell inclusions have already been described by various authors. The fact that these particular sporozoans are among those described by Joyet-Lavergne (1926) in a comprehensive paper added further interest to the study.

I wish to thank Professor J. Brontë Gatenby for his suggestion of this problem, and for his invaluable advice at all stages of the work.

MATERIAL AND TECHNIQUE.

The gregarines parasitic in larvae of T e n e b r i o m o l i t o r L. are, in many ways, ideal material for study by this method. The mealworms are cheap, and easy to obtain; they can be kept and fed with little difficulty; they have quite a high percentage

of infection by the gregarines; they fit well into the centrifuge rotor, and so can be centrifuged alive.

Against all this there is one drawback: it is impossible to tell whether the mealworm is parasitized or not, until the whole process of centrifuging, fixing, embedding, and sectioning has been completed. But, by examining the wax sections under the microscope, it is usually easy to see if parasites are present or not, without staining and mounting the sections permanently.

Another difficulty is that of getting good fixation of the parasites, for the fixing fluid has to penetrate the thick gregarine pellicle as well as the cells of the gut-wall of the host. This, however, may be minimized by cutting the gut into very small pieces.

The Tenebrio larvae containing the gregarines were sent from London, and conveniently kept in tins with perforated lids. They were fed on crushed oats.

Many different fixatives were tried, and it was found that the methods of Champy, Benoit, and Altmann (Baker's modification) gave a satisfactory fixation of the mitochondria. The material may then be stained by the iron alum haematoxylin long method, by the acid fuchsin picric acid method of Altmann, and by the Bensley Cowdry modification of it.

The Golgi material is best shown by the osmic acid fixation method of Weigl or Mann-Kopsch. The long fixation method advised by Baker, in Mann's fluid gives good results. After five to six days in osmium tetroxide at 30° C., the material is left in distilled water at the same temperature for 24 to 36 hours. Silver methods, such as those of da Fano, Cajal, and Aoyama, give no satisfactory results in spite of repeated experiments varying the times of immersion in the fixatives, silver nitrate solution, and reducing liquid. The Aoyama cadmium-chloride method, which gave beautiful and consistent results in the cells of the host gut-wall, gave no good results in the gregarines.

The fat is preserved by the osmium tetroxide-containing fixatives, and becomes a brown colour. It is also very well shown by the Sudan IV technique after formol saline fixation, recommended by Kay and Whitehead (1935). The paraglycogen is very stable and is beautifully demonstrated by the iodine

gum method advised by Langeron. It provides a very useful way of determining quickly whether parasites are present on a slide or not, for the dark brown colour of the paraglycogen granules is immediately visible.

As well as the more usual nuclear stains, Feulgen's nuclear reagent was tried with interesting results. When Champy and Benoit fixed sections were used, the long hydrolysis advised by Painter in the new edition of the 'Microtomist's Vade-Mecum' (1937) was necessary.

When centrifuged material was being prepared for cutting wax sections, the three or more centrifuged guts were embedded in one block, while several other uncentrifuged guts were treated in exactly the same way and embedded in another block, as controls. These wax sections were cut $2\cdot5\mu$ thick. The gregarine material was also studied in fresh smear preparations.

Centrifuging Technique.

Although the centrifuge top or rotor will actually hold six or seven mealworms, three is the maximum number that should be centrifuged at one time. This allows them enough room to lie in one row along the outer edge of the cavity; if they lie two or more deep, the worms nearer the centre will be subject to a lower centrifugal force than the outermost ones.

Various forces and times were experimented with, and it was found that at high pressures the gut was often torn out of the worm, breaking at one end (usually the anterior) and remaining attached at the other. The parasites themselves, on account of their thick pellicle, stand the pressure very well.

When they have been sufficiently centrifuged—three or four minutes at a pressure of 40 lb. per square inch is usually long enough—the worms are quickly removed from the rotor, the head and last millimetre or two are cut off with scissors and the gut carefully drawn out with forceps. It is best to do this from the hinder end of the animal, so that there is no danger of breaking the anterior region of the mid-gut during the process. It is cut in the region of the Malpighian tubules and the mid-gut part is put, at once, into the fixative, where it is cut into small pieces.

The importance of cutting it up into very small parts, or teasing it with a pair of needles, must be stressed, because most of the fixatives containing osmium tetroxide have poor powers of penetration, and it is imperative that the gregarines should be fixed as soon as possible.

A generous volume of the fixing fluid should also be used. When experimenting with different centrifugal forces, smear preparations were found very useful, as one can quickly see, without fixing or staining whether sufficient force to move all the paraglycogen granules down to the heavy pole, has been applied or not.
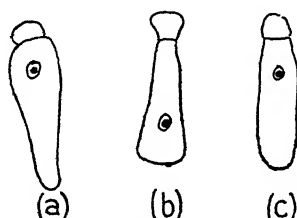
TEXT-FIG. 1.

Diagram of the three species found. (a) Gregarina steini; (b) Gregarina cuneata; (c) Gregarina polymorpha.

OBSERVATIONS.

Three different gregarine species were found in the gut of the Tenebrio larvae (see Text-fig. 1). At first, I supposed these to be the three species described by Léger and Duboscq in 1904 and Joyet-Lavergne in 1925, namely Steinina ovalis, Gregarina polymorpha Hamm, and Gregarina cuneata Stein, but after reading the relevant literature I believe that Steinina ovalis seldom or never parasitized the mealworms I used, but that Gregarina steini is very common. This question of identification is discussed at greater length below (see p. 315). However, since the cytoplasmic inclusions. and their behaviour when ultra-centrifuged, seem to be similar for the three species, the following account may be taken as general except where it is stated to be specific.

Five different kinds of inclusions have been found in the

cytoplasm of the gregarines studied, and these have characteristic positions in the centrifuged animal (see Text-fig. 2).

The lightest bodies are the fatty globules; these go to the extreme centripetal pole of a centrifuged gregarine and share this position with the Golgi material existing in the form of small granules, when this is present. Next come the larger Golgi bodies as irregular platelets and rods. The nucleus takes up a position below the Golgi apparatus and is underlain and
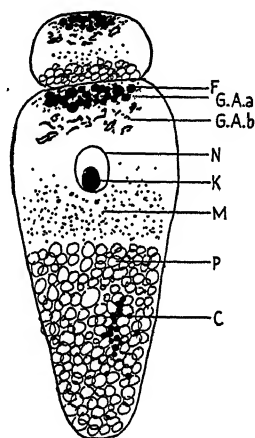
TEXT-FIG. 2.

Generalized diagram of a centrifuged gregarine. *F.*, fat; *G.A.* (*a*) granular Golgi material; (*b*) larger Golgi elements; *N.*, nucleus; *K.*, karyosome; *M.*, mitochondria; *P.*, paraglycogen. *C.*, chromidia.

surrounded by the mitochondria. Paraglycogen granules have a greater specific gravity than the mitochondria and go to the centrifugal pole. Chromidial granules may be seen in this region, among the paraglycogen bodies, and have, apparently, a specific gravity very similar to that of the paraglycogen.

Fat.

Fatty globules are found in all species, but the amount present varies, apparently, ontologically and in different species. The globules themselves may also vary very much in size, and in an uncentrifuged gregarine they are scattered irregularly

mitochondria and the Golgi material. He believed that there was only one such type of inclusion, and that it represented a primitive condition in which the mitochondria and Golgi elements had not yet been differentiated.

Joyet-Lavergne, and Duboscq and Grassé, however, do not agree with him and say that they are able to distinguish between the Golgi bodies and the mitochondria by careful fixing and staining. It seems possible that Hirschler's preparations may only have shown the granular Golgi material, which morphologically is very like the mitochondria, but the ultra-centrifuge shows clearly that there are two kinds of inclusion with different specific gravities.

Golgi bodies have been described in several sporozoans since then—King and Gatenby (1923) in Adelea ovata, where the apparatus is juxta-nuclear in young sporozoites and merozoites but spreads throughout the cell during ontogeny.

Tuzet (1931) finds a similar behaviour in the Golgi bodies of Gonospora duboscqui, and so does Joyet-Lavergne (1925) for Aggregata eberthi. The other coccidian studied by him has a typical Golgi apparatus. Dobell (1925), however, who has made such a thorough study of Aggregata eberthi is not convinced that it possesses a Golgi apparatus. Joyet-Lavergne finds that in the microgametes and sporozoites of Aggregata eberthi, the Golgi material is very closely connected with the nucleus, and he suggests its homology with the Golgi apparatus of metazoan spermatids. In the microgametes of the gregarine Nina gracilis he finds the Golgi apparatus in a similar position.

Hatt (1931) also describes osmiophilic Golgi platelets in the cephaline stages of the porosporid gregarines—Porospora gigantea in the gut of the lobster, and Nematopsis legeri in the gut of the crab.

In fixed and stained sections, cytoplasmic differences have often been described between the male and female sporozoan parasites. Léger and Duboscq in 1909 published a very important paper on the cytoplasmic differences of the sporonts of many gregarine species. Dobell is able to distinguish between male and female gametocytes of the coccidian Aggregata

eberthi very early in their ontogeny. Joyet-Lavergne has studied both gregarines and coccidians and finds several general differences between the male and female cytoplasm; these are: more Golgi material, more numerous and basiphil mitochondria and smaller alveoli in the male than in the female. Also, lipoids alone are found in the male, but lipoids and neutral fats in the female.

As I did not work out the life-histories and stages of the Tenebrio gregarines, I cannot add much to this discussion, except that in some smears, very obvious differences in cytoplasmic staining were seen in pairs of gregarines in syzygy. One pair of Gregarina steini stained with borax carmine showed striking differences.

D. Mühl (1921) was the first cytologist to record these differences in living material stained with vital dyes, such as neutral red, Bismarck brown, methyl violet, and methylene blue. She gives striking colour plates showing the quite different staining of the primite and satellite of Gregarina cuneata syzygy pairs. She thinks that these colour results are probably due to pH differences in the male and female cytoplasm.

Joyet-Lavergne (1926) stained the same parasites vitally with neutral red and also found differences, but he thinks that they are, rather, due to rH (oxido-reduction potential) differences of the gregarines, the female having a less intensive rH than the male.

P. Rey has recently (1931) gone over this work of Joyet-Lavergne, using quantitative rather than qualitative methods, and finds that in Gregarina polymorpha the pH and the rH are similar in the male and the female parasites. He cannot, however, give any indication regarding the causes of the admittedly observed sexual differences. Joyet-Lavergne is not convinced and believes that the methods used by Rey are too crude to estimate the pH differences, which are very small but still are present.

The actual bodies stained by the vital dyes must now be discussed. Mühl found that there are globules in the cytoplasm which have a great affinity for the neutral red dye and rapidly become saturated with it. She thinks these globules are probably concerned with enzymes and digestion.

The rate of penetration of the dye varies in the two sexes of Gregarina cuneata. It also varies in the species studied; for example, she found that while she could well observe the granules in the cytoplasm of Gregarina cuneata for an hour or more before the colour became diffused throughout the cell, the cytoplasm of Gregarina polymorpha became generally coloured by the dye almost at once.

Joyet-Lavergne (1926) obtained different results when he put the gregarines into a neutral red solution, for the first cytoplasmic bodies to take up the red colour were the Golgi elements, in the form of 'petits arcs ou granules'. Then he found a general diffuse colouring of the gregarine to take place, with the appearance of the globules stained by Mühl. However, 'il est parfois difficile d'établir la distribution exacte des éléments en arcs colorés au rouge neutre, car cette couleur ne reste pas très longtemps localisée à ces éléments; elle diffuse dans le cytoplasme pour donner finalement les phases de colorations des globules étudiées par Mühl'. He thus agrees with Parat, in finding a Golgi apparatus stainable in neutral red. No cytologists now believe that the Golgi apparatus proper can ever be stained by neutral red.

Another French cytologist takes a contradictory view to that held by Joyet-Lavergne. O. Tuzet was able to stain Gonospora duboscqui with vital dyes and get the following results: mitochondria are present and are stained vitally with Janus green, and larger globules are present which stain with neutral red solution (1 in 10,000). These, in young forms, are found near the nucleus, but as growth proceeds, become scattered throughout the cytoplasm. These form the 'vacuome'. A third category of bodies is seen; these will stain neither with neutral red nor Janus green, but with osmic methods, by which they are seen to be much larger and fewer in number than the neutral-red bodies or 'vacuome' granules. These Golgi bodies, like the neutral-red bodies, are situated near the nucleus in young parasites and in older ones throughout the cell.

I made some smear preparations of the mid-guts of the mealworm larvae and examined them in a physiological salt solution of neutral red. Globules of a red colour appeared in the cyto-

plasm of Gregarina cuneata and Gregarina steini, and after some time the red colour diffused throughout the whole parasite, making it impossible to distinguish separate granules. This is as described by both Mühl and Tuzet. These globules were never rod-like, as described by Joyet-Lavergne.

Some of the gregarines were centrifuged and then examined as before in the neutral red solution. Red globules appeared as before, and irregularly throughout the cytoplasm, apparently in no relation to the other inclusions, which were banded.

This is rather a strange result, because if the granules had been pre-existent in the gregarine, one would have expected them to have been moved one way or another when the gregarine was centrifuged. There may, however, be special areas in the cytoplasm which take up the stain well, but are not disturbed by the centrifugal force which moves the paraglycogen granules. On the other hand, the granules may have been formed in the cytoplasm, after the centrifuging had taken place—although if they arose in connexion with any of the other banded inclusions, one would expect to find them in the relevant area.

The question of the function of the Golgi apparatus is an interesting one. It is usually believed to be in some way concerned with secretion. In metazoan spermatogenesis it has a very important and peculiar function—that of being responsible for the acrosome formation.

As already mentioned, Joyet-Lavergne describes a group of Golgi elements connected closely with the nucleus of the microgamete and sporozoite of Aggregata eberthi and suggests a homology with metazoan spermatogenesis. It should be noted clearly that in the Metazoa, although the Golgi apparatus is associated in some way with acrosome formation, it does not metamorphose directly into it, and in a mature spermatozoan the Golgi apparatus has moved right down to the proximal end of the cell, leaving the acrosomic cap resting distally on the nucleus.

The parabasal body of Mastigophora, which Duboscq and Grassé take to be a primitive type of Golgi apparatus, is also

known to be secretory in several cases. In B o d o and C r y - t o b i a it secretes glycogen and in J o e n i a and T r i c h o - m o n a s, other substances. They take this as evidence that the parabasal body is the homologue of the Golgi apparatus.

Joyet-Lavergne thinks that there is probably some relation between the Golgi apparatus of the Sporozoa and the formation of fatty substances; this, I think, is possible.

Nassonow's theory that the contractile vacuole of ciliates is homologous with the Golgi apparatus of other animals, attributes to the apparatus an excretory function. At present, however, the theory does not find many supporters, especially after the work of H. W. Beams and King; see also R. Brown's paper on P a r a m o e c i u m (communicated).

Mitochondria.

In a centrifuged gregarine mitochondria are seen immediately proximally to and surrounding the nucleus; in a control preparation they are scattered through the cytoplasm (fig. 5, *b*, Pl. 28). They appear typically as small granules, but in some of the fully grown sporonts short rod-like mitochondria may be seen.

In a centrifuged animal the mitochondria take up a position centripetal to the paraglycogen, between it and the nucleus. One gut which was heavily parasitized with young stages of G r e g a r i n a s t e i n i showed especially beautifully the mitochondria as an aggregation of fine granules in this position (fig. 3, Pl. 28). Young stages are more satisfactory than older ones for studying the effect of the centrifugal force on the cytoplasmic inclusions, as they have fewer paraglycogen granules and so the inclusions that go to the lighter pole have more room to become arranged in their characteristic positions. In adult gregarines there is so much paraglycogen that little room is left for the other bodies which have, therefore, to lie very close together and so their proper positions are difficult to make out.

There has been much less controversy about the mitochondria of Protozoa than about their Golgi apparatus, and there is almost unanimity of opinion as to their universal occurrence.

Fauré-Fremiet discovered mitochondria in ciliates in 1910, and

this was the first time they were found in Protozoa. Hirschler followed by showing that they occurred in gregarines as well, in 1914.

Typical mitochondria have been found, I think, by all cytologists studying the Sporozoa, with the exception of Hirschler for Gregarina polymorpha, as before mentioned.

There is remarkably little known of the exact function of the mitochondria in the cell. As they are of almost universal occurrence in plant and animal cytoplasm it is assumed that they play a fundamental part in the life of the cell. Cowdry says that in embryology they are associated in the formation of certain substances like fat, lipoid, pigment, and perhaps also secretion granules, and remarks that these substances are not formed by direct chemical transformation of the mitochondria, but that the exact part played by them is unknown.

The work of Hirsch and Duthie supports this secretion theory, as they found that the zymogen granules in the pancreas arise in connexion with the mitochondria.

Horning believes that they are in some way concerned with intracellular digestion. Cowdry also suspects that the mitochondria 'are concerned, directly or indirectly, with processes of metabolism or protoplasmic respiration'. This was suggested by his work with vital stains, and he says that the Lewises get similar results from their tissue cultures.

Joyet-Lavergne believes that for the gregarines he describes, there is a connexion between the mitochondria and the oxidizing glutathione, and relates the mitochondrial differences in the male and female gregarine cytoplasms to his observed rH differences in the individuals of a syzygy pair.

Chromidia.

There has been so much discussion over the meaning of the word 'chromidia', that I use it with hesitation, and only in the sense that it indicates the nuclear origin of the bodies so called. These granules seem to correspond to the 'réserves albuminoides' of Joyet-Lavergne. As, however, they differ apparently from the bodies he describes in not giving Millon's reaction and in not having a mitochondrial element attached to them, I think it is best to use the old name, chromidia.

In one centrifuged gut containing young Gregarina steini stages, almost every parasite had a group of these chromidia, which took up a position in the paraglycogen region (see fig. 3, Pl. 28). However, they were much less often seen in adult gregarines, where they also vary more in size.

Although they give a strong colour with haematoxylin staining, as does the karyosome, they give a negative result with Feulgen's nuclear reagent, showing the absence of thymonucleic acid, which is normally a constituent of chromatin. This result is of interest in connexion with the Feulgen results given by the nucleus to be discussed later. There can be little doubt that the granules are extrusions from the karyosome. Buds are seen on its surface, they are again seen in the nuclear sap and then in the cytoplasm near the nucleus, but how they penetrate the nuclear membrane is unknown. This nuclear extrusion will be discussed below.

Chromidial granules have been described in sporozoan, as well as other protozoan cytoplasm by many authors. Joyet-Lavergne thinks that they are all probably homologous with his albuminoid reserves; for example, Dobell describes 'granules and small lumps of a material closely similar to, or identical with, chromatin'—and also 'granules of a similar substance—probably volutin' in the cytoplasm of Aggregata eberthi, and Joyet-Lavergne calls these bodies albuminoid reserves when working on the same coccidian. He believes that the chromatoid bodies in the gregarine cytoplasms described by Léger and Duboscq, Schneider, Léger, Ceccioni, and Drzwiecki are all similar and can be homologized with his albuminoid bodies.

Volutin is another substance that has been described in protozoan cytoplasm. It seems to be related to chromatin in composition and is said to stain with nuclear stains. It is confined to protozoan cytoplasm and has not been demonstrated in Metazoa.

The characteristic test for volutin is methylene blue followed by treatment with a 1 per cent. solution of sulphuric acid which removes the colour from everything except the volutin.

When this test is applied to smear preparations of the Tenebrio gregarines which have been fixed in Schaudinn's

fluid, blue granules appear in the cytoplasm. When centrifuged animals are examined after the same treatment, it is seen that the blue granules have moved down to the centrifugal pole of the gregarine and are placed among the paraglycogen granules. That these are volutin granules seems probable, both from the fact of their characteristic staining and from the fact that Patten and Beams found granules in a similar position among paramylum granules, in centrifuged Euglenae. They believe these bodies to be volutin granules. On the other hand, if these are volutin granules, one would expect to find similar bodies in a Champy iron alum haematoxylin preparation. Now the chromidia do occur in this position, in Gregarina steini at all events, but the granules seem fewer in number than those got by Meyer's methylene blue method. It seems, therefore, that there is some close relationship between these two types of cytoplasmic inclusions.

Other writers have made like suggestions; Reichenow believes that they are essentially similar, and Joyet-Lavergne (1926) says 'Il n'est donc possible de dire si cette formation (volutin) est un élément distinct de ceux qui ont été décrits ou si, au contraire, elle réalise simplement un aspect particulier de l'un des constituants du cytoplasme, par exemple, des chromidies.'

Paraglycogen.

Paraglycogen is the substance in the gregarines studied having the greatest specific gravity, and in a centrifuged parasite it is found at the centrifugal pole.

The name 'paraglycogen' was used first by Bütschli in 1885, and Maupas described the same material a year later as 'zoo-amylon'.

It has often been described in the Protozoa but never in the metazoan cells. Like glycogen, paraglycogen gives a dark brown colour with iodine, but differs from it in several important ways histologically: (i) it is undigested by amylase of the saliva; (ii) it is insoluble in water; (iii) it gives a negative result with Best's carmine, which is a well-known test for glycogen.

The iodine gum method recommended by Langeron was found

very useful. Preparations are quickly and easily made, and have the advantage of being permanent, and it is possible to tell at a glance the number of parasites present in a gut section (p. 3).

The granules are usually regularly disc-shaped, but may be more or less distorted. The discs seem to have a central depressed area, which as the 'hile' is described in detail by Joyet-Lavergne.

In control gregarines the paraglycogen granules lie in the centres of the 'alveoli', which are formed by the coagulation of the cytoplasm around the granules. In centrifuged gregarines the lighter cytoplasmic pole which is free of paraglycogen granules presents, usually, a homogeneous non-alveolar appearance. Young gregarines have their paraglycogen in the form of small granules, while in adults it is found as large and small ones.

It is interesting to note that the paraglycogen area in a centrifuged parasite, treated by Feulgen's method for the nuclear reaction, gives a pink colour. The granules are not separately stained but there is a general diffuse stain in the centrifugal region. This result is not due to stale Schiff's reagent being used and the fuchsin acting as a stain, for the same colour was got with the freshly prepared reagent.

Bauer's technique for demonstrating glycogen was tried. Gregarines were fixed in Bouin-Allen, post-chromed and then treated with Schiff's reagent without previous hydrolysis. Again, the paraglycogen area usually became a pink colour, but in some cases the actual granules were separately and distinctly coloured, giving what might be called a typical glycogen test. Lison (1936), however, does mention the fact that starch, galactogen, and other substances give a positive result as well as glycogen.

Nucleus.

The nucleus of these gregarines is typically 'karyosomic', having a large spherical body, staining densely with haematoxylin, in the non-chromatic nuclear sap. This karyosome moves through the sap to the centrifugal nuclear pole in a centrifuged gregarine. Sometimes a stratified appearance is seen in the karyosome itself, apparently separating a light

plasmatic material from the heavier chromatic substance. An interesting nucleus is drawn (fig. 7, c, Pl. 28) in which there is a banding of the karyosome and of the bud about to be given off from it. This leads on to the important question of nuclear extrusions. Many karyosomes, control and centrifuged, show the formation of buds which drop into the nuclear sap and are eventually released into the cytoplasm, getting out through the nuclear membrane by some means. They arise, usually singly, from the karyosomic periphery and the process could not possibly be interpreted as mitotic division. Various stages in the process are shown in fig. 7, Pl. 28.

While the oocytes in Saccocirrus provide a very well-known example of nuclear extrusion in the Metazoa, similar extrusions have been known to occur in sporozoans for a long time. Marshall described the expulsion of chromidia at the end of the last century and Berndt at the beginning of the present. Léger and Duboscq in 1904 also showed that this process takes place in the nucleus of several gregarines, e.g. Stylorhynchus longicollis, Stylorhynchus oblongatus, and also species of Stenophora. It occurs when the gregarines are still attached epimeritically to the host-cells, and when the granules reach the cytoplasm they are described as chromidia, and would now be called albuminoid reserves by Joyet-Lavergne.

Paehler (1904) gives drawings of apparently the same process in Gregarina ovata. Brasil (1909) shows similar karyosomic budding with granules in the cytoplasm for Selenidium mesnili. Pfeffer (1910) described 'geflammte' nuclei in the Tenebrio gregarines. These have a very irregular nuclear membrane, and a nuclear sap with many chromatic granules evidently of karyosomic origin. The vacuolated appearance of the karyosome described by him was sometimes seen in my gregarines (fig. 7, g, Pl. 28).

Pfeffer finds karyosomic budding to occur particularly vigorously at the time of sexual association, and believes the 'geflammte' nuclei to be characteristic of this period. I had made some very similar preparations, but had thought this type of nuclear appearance due to bad fixation. This seems a simpler

explanation of a whole gut full of gregarines with 'geflammte' nuclei than that they are all preparing for association.

Paehler also shows a nucleus that is very irregular in outline, and completely chromatic; again I would have explained it by bad fixation.

From its staining reactions with haematoxylin, one would call the karyosome chromatinic, but when the Feulgen nuclear reaction is used, the karyosome gives a negative result for the presence of chromatin, or rather thymonucleic acid, a normal constituent of chromatin; neither are the purplish red granules present in the nuclear sap. These results cannot be explained by faulty methods of technique, for the nuclei of the host tissue exhibited the chromatin very satisfactorily.

This is a very unexpected result, and a similar one is found when the cytoplasmic chromidia are examined; neither they nor the karyosome show the typical purplish colour. One would not be very surprised at an absence of thymonucleic acid in the chromidia, for, even though of karyosomic origin, they could undergo a chemical change in the cytoplasm, but it is unusual to find nuclei with apparently no chromatin. However, it is very interesting to note that both Ludford for Limnaea stagnalis, and M. S. Gardiner for Limulus (1935) found that developing oocytes pass through a stage where a negative result is given by the nuclear reaction of Feulgen. Also, Patten found that in two of the protozoans studied by her, Merocystis kathae (1935) and Piridium sociabile (1936), similar negative results were regularly obtained in certain stages. Alternative explanations are given by Gardiner: either (a) the thymonucleic acid and, therefore, the aldehyde formed by its hydrolysis, is very finely dispersed in the nucleus and so the resulting red colour after Schiff's reagent is impossible to see, even with an oil immersion; or (b) the thymonucleic acid has undergone some chemical change and is not hydrolysed by the acid with aldehyde formation.

Both Ludford and Gardiner believe that the former is the correct explanation—possibly a similar explanation holds for the Tenebrio gregarines.

The aceto-carmine chromatin test also gives a negative result.

Some general observations may now be noted. The parasites are never found in the hind-gut of the larvae, but in the mid-gut anteriorly. The pupal and the adult T e n e b r i o beetles were never found to be infected. This agrees with the observations of Pfeffer, but he did once find a doubtful parasite in a beetle gut. He also notes that after keeping the T e n e b r i o larvae for some time the degree of infection becomes less; I found the same thing in my larvae, which seem to become more immune as they get older—certainly they have ample opportunity of ingesting the cysts from other larvae, with so many living in the same tin. This decrease in infection is not to be explained by any selection of the worms killed, as they were taken quite at random from their tins. It occurred to Pfeffer that a second host might be necessary in the life-cycle, but after experiments with rats and mice he thought it unlikely. Such a thing is very rare in gregarines, but the porosporids provide a good example of its occurrence. P. Hatt distinguished between two genera, P o r o s p o r a and N e m a t o p s i s which have gymnospore stages in the gills of Molluscs and free adult stages in the gut of Decapods.

Although usually in fresh preparations made of centrifuged guts and examined at once, the parasites are motionless, if a little centrifugal pressure is used gregarines may be seen actually moving, their cytoplasmic inclusions being in the stratified state.

A peculiar occurrence was noted two or three times; after slow centrifuging, pairs of gregarines in syzygy were seen moving around in a circle; this continued for a few minutes, and then they straightened out and proceeded normally in an approximately straight line.

Occasionally, also, in smear preparations individuals were seen in which the centripetal pole of a syzygy pair was at the anterior end of one, and posterior end of the other partner, or vice versa. The probable explanation is that the partners were lying side by side preparatory to encystment when centrifuged, and became straightened out in the process of smearing the gut, remaining attached head to tail.

Preparations were made to test for the presence of Vitamin C by the silver nitrate acetic acid technique given by Bourne in

the new edition of the 'Microtomist's Vade-Mecum' (1937). The whole mid-gut was fixed, washed, and reduced in photographic 'hypo' in a dark room, and then teased up and examined in glycerine. The gut cells showed black granules which may be Vitamin C, but the parasite cytoplasm was quite clear. A similar negative result was obtained when smear preparations were treated.

Large granules appear characteristically in the protomerite of Gregarina steini. They are well preserved by Dobell's, Bouin's, and Schaudinn's fixatives. They become a red colour in Giemsa's stain, and are blue in methylene blue methods. The blue colour is remarkably resistant to sulphuric acid. They are not stained by the iron alum haematoxylin method. They sometimes take up the fuchsin colour after Feulgen's nuclear test, and move towards the heavy pole in a centrifuged animal. They do not stain brown with iodine, but become red after Bauer's method. They are also figured by Mühl (1921). They are possibly related in some way to volutin or chromidial granules.

Sections of larval gut show very peculiar inclusions in the nuclei of the basal cells. These are quite crystalline in appearance and are either rectangular or flat hexagonal plates. They stain deeply with the iron alum haematoxylin method, but give a negative result with Feulgen's nuclear reagent. They take up the fuchsin colour in Altmann's stain, but part with it readily on differentiation with picric acid. They are not darkened by osmium tetroxide. These bodies have been described by Pfeffer, but are not mentioned by Gardiner in her work on the nucleolus of Tenebrio cells.

The cells of the mid-gut are also of interest for the cytoplasmic prominences which project into the lumen. These may look like Flagellates, the pear-shaped or spherical body being attached to the gut by a narrow cytoplasmic connexion. They contain no nuclei, but have osmiophilic and haematoxylin staining globules in their cytoplasm, and are probably concerned with secretion.

DISCUSSION.

Joyet-Lavergne differentiates between two kinds of fatty substances 'des lipoïdes et des graisses'. I did not try all the methods he advises, because several of them, such as nile blue, Ciaccio, and the old Sudan III technique, are now considered to be unspecific by the German workers. However, it seems probable that there is more than one kind of fatty substance present in these gregarines, as different degrees of darkening are observed in the fatty globules after treatment with osmium tetroxide. The permanence of this colouring also varies. This may, however, depend upon the original degree of osmication of the globules.

Joyet-Lavergne also notes that the male cytoplasm has in general a much greater proportion of Golgi material than the female, in the sporozoans he studied. Certainly, in respect of the Tenebrio gregarines, the difference in the amounts of Golgi material present in the cytoplasms of different individuals is very striking. Whether these are due to sexual differences or not, I can offer no opinion.

Regarding the mitochondria, Joyet-Lavergne has noted the fact that the young individuals always have granular mitochondria, but the older ones have both granular and rod-like bodies. In the gregarine cytoplasms that he has drawn, the rod-like mitochondria—'chondriocontes'—are more frequent than I found them, but his plate of drawings is of the two coccidians Aggregata eberthi and Adelina dimidiata, and the gregarines Nina gracilis and Stylorhynchus longicollis, and not of the three gregarines from Tenebrio. He also states that the 'chondriocontes' are very often attached to a globule of 'réserve albuminoide'. He finds these latter inclusions to be normal constituents of sporozoan cytoplasm and believes that they and the mitochondria connected with them are of nuclear origin. These albuminoid reserves must correspond to the chromidial granules I found in the Tenebrio gregarines; like his, these stain with nuclear stains and are usually larger than the mitochondria, and like his, I believe, they have an origin in the nucleus; but I did not find

any mitochondria forming little caps on them, and neither could I get a positive reaction with Millon's test, which he finds so characteristic.

Although I was unable to show the presence of Vitamin C in these gregarines, it has been described as occurring in other Protozoa, such as Chlamydomonas and some ciliates. The vitamin may be found either in the reduced or in the oxidized form, and the test I used is only for the reduced type, so it is possible that the oxidized vitamin is present in the gregarines.

Glycogen does not seem to occur in the gregarines examined, although sometimes the paraglycogen area in a centrifuged animal gives a red colour when treated with Bauer's technique. However, Lison states that this test is not specific for glycogen. When gregarines are treated with Best's carmine no glycogen in shown. Joyet-Lavergne obtained similar results for all the Sporozoa he examined (1926), but Dobell (1925) claims to have been able to show glycogen as well as paraglycogen in Aggregata eberthi.

With regard to the identification of the gregarines studied, I was able to distinguish between three species in smear preparations with characteristic shapes (Text-fig. 1), and of which Giemsa stain gave good differential colouring: (b) must be Gregarina cuneata from Léger and Duboscq's 1904 description, and that of Mühl 1921; (a) cannot be regarded as either Steinina ovalis or Gregarina polymorpha described by Léger and Duboscq.

However, diagrams 13 and 14 in Pfeffer's paper probably represent my species and these he calls 'Berndt's Gregarina steini' (referring to Berndt's 1902 paper) in the text. Two other species are drawn but not named, but they are probably Gregarina polymorpha and Gregarina cuneata. Although he discusses Steinina ovalis in the text, the gregarines shown in his Pl. 3 are not named and so I do not know if it is shown or not.

D. Mühl finds four species in the larvae from Marburg, the three described by Léger and Duboscq and Gregarina steini, but does not state how she distinguishes them. Her

Fig. 21 for Gregarina steini is exactly like (a) species, which I will, therefore, call Gregarina steini.

(c) is probably Gregarina polymorpha and is figured as such by Mühl, Figs. 23 and 24. On the other hand, her Fig. 30 labelled Gregarina polymorpha, shows eosinophile granules in the protomerite which is typical of my Gregarina steini after Giemsa staining. There may be some relation between Gregarina steini and Gregarina polymorpha. This is also suggested by the occurrence of a darkly staining area at the distal end of the protomerite as shown in my fig. 3, Pl. 28, and Mühl's Fig. 23 of Gregarina polymorpha.

I do not know whether fig. 1, Pl. 28, represents Gregarina polymorpha or a rare Steinina ovalis. The protomerite and epimerite resemble Text-fig. 4 d of Steinina ovalis in Léger and Duboscq's paper, but its deutomerite is much more like that of Gregarina polymorpha. In their Text-fig. 3, Steinina ovalis has an almost globular deutomerite, which is also found in Steinina rotundata from bird fleas, Ceratophyllus (see Ashworth and Rettie, 1912).

On the other hand, S. Ishii (1911) says that Gregarina polymorpha has no epimerite.

For the purpose of this paper, I did not think it necessary to go farther into the question of identification, interesting as it is, and did not attempt to work out the complete life-history of each species, which would be a very lengthy undertaking.

Though it may sometimes be difficult to distinguish between gregarine species in smear preparations, it is often almost impossible to do so in thin sections, so in most cases an attempt was not even made. Even size is no criterion, as the parasites vary very much in size during ontogeny and also a section may be longitudinal, transverse, or anything between the two.

Summary.

A. Three gregarine species are found to inhabit the mid-gut of the mealworm larvae used: Gregarina cuneata Stein, Gregarina polymorpha Hamm, and Gregarina

steini Berndt. The often described Steinina ovalis is probably seldom or never found.

They live only in the mid-gut of larvae. They are never found in pupal or adult forms.

Gregarines have been seen moving when in a stratified condition.

B. The gregarine cytoplasm has five important inclusions, each having a characteristic position in a centrifuged animal (Text-fig. 2).

1. Paraglycogen.—This gives a dark brown colour with iodine, a pinkish general colour with the acid fuchsin of the Feulgen technique, and often a red colour with Bauer's reaction. It occupies the centrifugal pole of the centrifuged cell and is in the form of disc-like granules of varying size.

2. In young centrifuged Gregarina steini chromidial granules are seen in the paraglycogen area, and have, therefore, approximately the same specific gravity. They arise by karyosomic budding with the subsequent extrusion of these buds into the cytoplasm. They stain with iron alum haematoxylin, like the karyosome, and both give a negative result with the Feulgen test for thymonucleic acid. They probably correspond to Joyet-Lavergne's 'albuminoid reserves', but do not have the mitochondrial 'cap' he describes.

3. Mitochondria.—These are usually granular, but sometimes rod-like. They are seen between the 'alveoli' formed by the paraglycogen granules. They lie distally to the paraglycogen in a centrifuged parasite; they stain by the iron alum haematoxylin long method, after Benoit, Champy, or Altmann fixation, also with Altmann's fuchsin picric acid stain and the Bensley Cowdry modification of it.

4. The Nucleus is karyosomic, and the karyosome is moved to the centrifugal pole by pressure as is the nucleolus of metazoan cells. The nucleus shows budding of the karyosome. There is plasmatic as well as chromatic material in the karyosome, as shown by centrifuging. The nucleus gives a negative result with Feulgen's nuclear reaction, but chromatin may exist in a very dispersed condition.

5. Golgi Material.—This lies at the centripetal end of `

the nucleus. It is best shown by Weigl fixation. The large and regular Golgi elements are slightly heavier than the granular Golgi material, which may be compared with that of young oocytes.

6. **Fatty Material** lies at the extreme centripetal pole of the cell, in globules of varying size. It becomes brown or black after treatment with osmium tetroxide, and vivid cherry red with Sudan IV. It gives a negative result with the Schultz reaction for cholestrol.

C. Large globules are seen in the protomerite of G r e g a r i n a s t e i n i, eosinophile, sometimes fuchsinophile, and also staining with methylene blue. These move towards the centrifugal pole.

Methylene blue preparations show blue granules among the paraglycogen granules in the centrifuged animal. They are remarkably resistant to dilute sulphuric acid. They are possibly allied to volutin or chromidia.

Tests for the presence of Vitamin C yielded negative results.

Only the inclusions of the gregarines in the gut lumen were studied, and the complete life-cycles of the species were not followed out.

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EXPLANATION OF PLATE 28.

The magnification of figs. 3 and 4 is twice that of the other figures.

All the figures were drawn with the aid of squared paper and a squared apparatus in the eye-piece. Bausch and Lomb binocular microscope, Koristka $\frac{1}{15}$ in. semi-apochromatic oil immersion objective.

F., fat; *G.A.*, Golgi apparatus; *K.*, karyosome; *M.*, mitochondria; *C.*, chromidia; *P.*, paraglycogen. The arrows show the direction of the centrifugal force.

Fig. 1.—Gregarina polymorpha(?). Smear preparation. Formol saline. Sudan IV and haemalum. Uncentrifuged. Fat-globules scattered throughout cytoplasm.

Fig. 2.—Gregarina steini. Smear preparation. Formol saline. Sudan IV and haemalum. Centrifuged. Fat-globules at centripetal pole; karyosome at centrifugal pole of nucleus.

Fig. 3.—Gregarina steini. Champy. Iron alum haematoxylin. Centrifuged. Shows stratified effect. Paraglycogen granules not shown, but reach from mitochondrial region to extreme centrifugal pole.

Fig. 4.—Gregarina steini. Champy. Iodine gum. Paraglycogen (dark brown) at centrifugal pole and fat (black) at centripetal pole. Karyosome also moved.

Fig. 5.—(a) Champy. Iron alum haematoxylin. Part of cytoplasm. Uncentrifuged. Paraglycogen, fat, and a chromidium. Mitochondria are not shown. (b) Benoit. Iron alum haematoxylin. Part of cytoplasm. Uncentrifuged. Here mitochondria are seen, but not fat.

Fig. 6.—Champy. Iodine gum. Cross-section of young parasite—uncentrifuged, showing small paraglycogen granules (brown) and fat-granules (black).

Fig. 7.—Nuclei alone—All centrifuged except (*d*). All Champy and iron alum haematoxylin except (*a*) and (*b*) which are Benoit and iron alum haematoxylin and (*g*) which is Kolatchew.

Fig. 8.—Gregarina polymorpha(?). Weigl. Centrifuged. Line limiting paraglycogen area rather irregular. Golgi bodies at centripetal pole. No fat seen here.

Fig. 9.—Gregarina polymorpha. Weigl. Control—uncentrifuged, showing Golgi apparatus throughout cytoplasm, but fat globules only in protomerite.

Fig. 10.—Gregarina cuneata deutomerite(?) showing granular and larger Golgi bodies, in cytoplasmic area to centrifuged end of paraglycogen area.
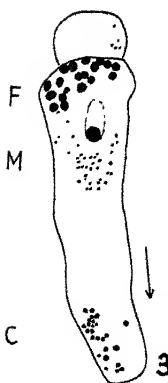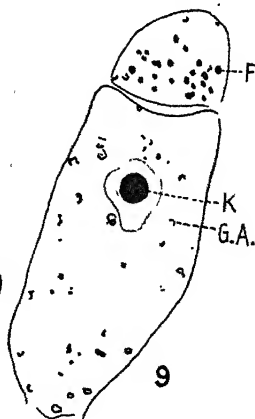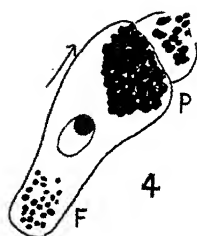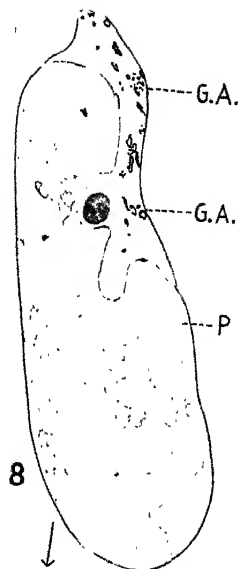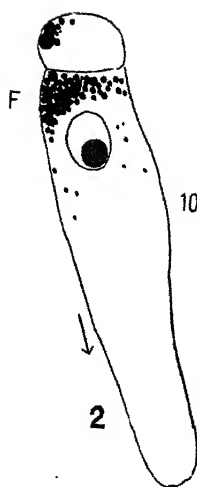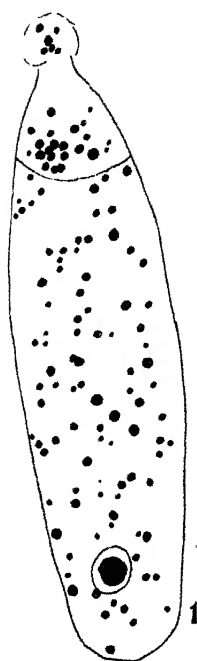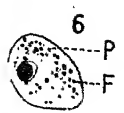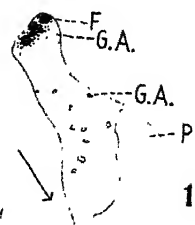
G.A.
G.A.
P

100 μ

F

P
F

F

K
G.A.

F
M
C

F

50 μ

(a) 7 (b)

(c) (d)

(e) (f)

F
G.A.

G.A.

P

P
F
C

P
M

P
F

(a) 5 (b)

6

(a)
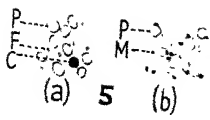
On the Ciliary Mechanisms and Interrelationships of Lamellibranchs.

PART V: Note on the Gills of Amussium pleuronectes.

By

Daphne Atkins, B.Sc.

Marine Biological Laboratory, Plymouth.

With 2 Text-figures.

CONTENTS.

INTRODUCTION.

Two specimens of Amussium pleuronectes L. from the Orissa Coast, Bay of Bengal, were obtained from the Indian Museum, Calcutta, through the courtesy of Dr. B. Prashad. This species was found to resemble Pecten (including Chlamys), not only in the general anatomy, but very closely in the structure of the gills. The specimens had been preserved in alcohol for museum purposes, and though the fixation was quite adequate for work on gill structure, it was too imperfect to show histology and ciliation with any exactitude.

Amussium pleuronectes, which lies on the right valve, as does Pecten, is evidently an active swimmer, for the adductor muscle is composed largely of striated fibres, there being only a narrow strip of smooth fibres about 3 mm. wide where the entire muscle is about 18 mm. wide. The proportion of the adductor occupied by striated fibres is even greater than in Pecten and Chlamys.

THE GILLS AND PALPS.

The disposition and structure of the gills of Amussium pleuronectes closely resemble those of Pecten and

Chlamys. Each gill is suspended by a membrane and has a considerable free posterior portion. The arrangement of the ctenidial muscles differs slightly from that in Pecten. The paired longitudinal muscles of the gill axes include striated fibres, as do those of several species of Pectinidae (Janssens, 1893; Dakin, 1909; Setna, 1930). Such striated muscle-fibres are found in organs performing a series of comparatively sudden movements, and it is most probable that the gill axes in Amussium pleuronectes, as in Pecten, are capable of rapid contraction, and that this occurs preparatory to the clapping of the valves in swimming.

The inner demibranch is rather deeper than the outer: the ascending lamellae are rather more than two-thirds the height of the descending. The lamellae are highly plicate and heterorhabdic (Text-fig. 1). The upper edges of the ascending lamellae are free from adjacent parts of the body. The upper ends of the ascending filaments are united in series by interlocking cilia to a depth of about a millimetre. At the lower edge of the demibranch adjacent filaments are connected by ciliated disks. The connexion between the fflaments is by interlocking cilia borne on long spurs, those of the principal filaments being especially long. At the greatest depth of the demibranch there are about twenty spurs on the descending filament and fifteen on the ascending. Chitin extends into the spurs both of the principal and ordinary filaments. Horizontal muscles (*h.m.*, Text-figs. 1, 2) are well developed in the principal filaments, the arrangement closely resembling that in Pecten; it is extremely probable that Amussium pleuronectes can also flap the sides of these filaments (see Text-figs. 1 and 2).

The interlamellar junctions have the form of low septa extending about two-fifths of the height of the principal filaments. An interlamellar extension, or respiratory expansion (*r.e.*, Text-fig. 1), occurs on about the upper third of the descending principal filament; it appears to be of much the same structure as in Pecten.

An intrafilamentar septum, formed of muscle-fibres, is present in the filaments, and the chitinous lining of the ordinary filaments is thickened under the insertion of the fibres.
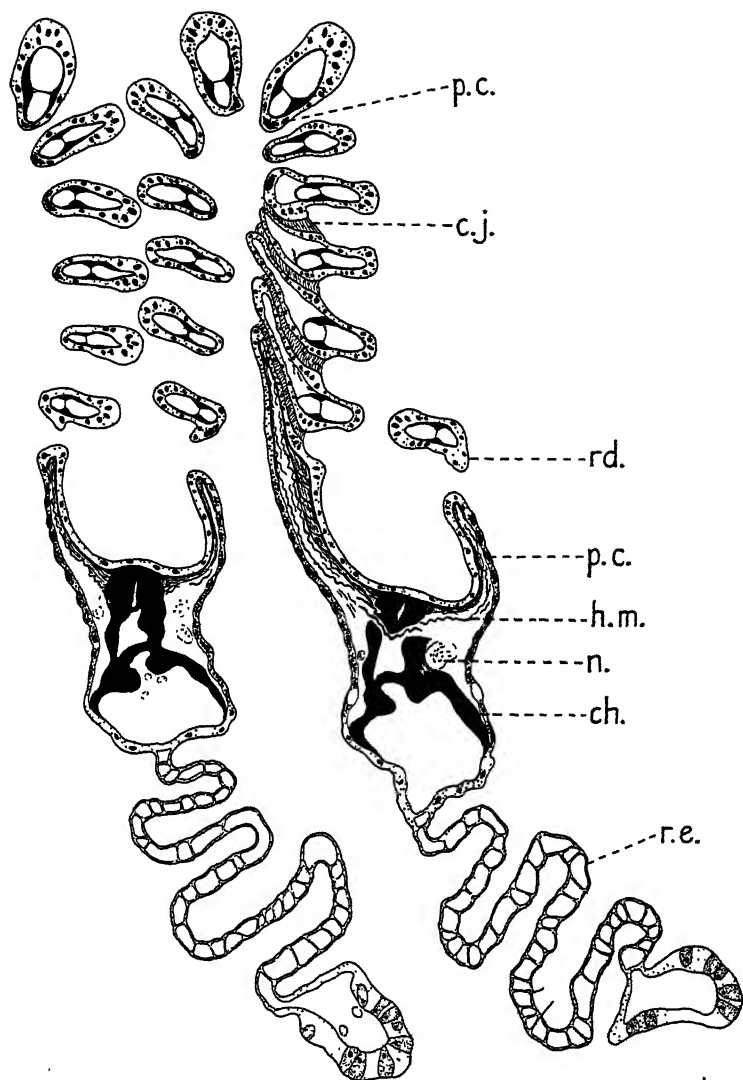
TEXT-FIG. 1.

Amussium pleuronectes. Transverse section through the
dorsal region of a descending lamella, showing a plica and a half,
and two principal filaments with respiratory expansions. *ch.*,
chitin; *c.j.*, ciliary junction; *h.m.*, horizontal muscles; *n.*, nerve?;
p.c., pigment cells; *rd.*, ridge on filament adjacent to principal
filament for interlocking with it; *r.e.*, respiratory expansion.
Alcohol fixation; Mallory's triple stain. ×168.

There are eleven to thirteen filaments, but usually twelve, to a plica; the plicae are deep in the upper part of the demibranch but tend to flatten out toward the free margin. The principal filaments are broad, except toward the lower edge of the demibranchs where they become almost indistinguishable from the ordinary filaments in surface view of the lamella. The difference in the form of the principal filament in different parts of the lamella, that is, with the frontal surface concave (Text-fig. 1) and convex (Text-fig. 2) is largely, or entirely, due to the action of the horizontal muscles (*h.m.*). In the upper part of the demibranchs, however, where there is little space for movement of the plicae, the frontal surface of the principal filaments is probably always or generally concave, as in Text-fig. 1. The form of the chitinous skeleton may be seen from Text-figs. 1 and 2.

Setna's (1930, p. 376) words concerning the structure of the principal and adjacent ordinary filaments of P e c t e n may be repeated here as applying to those of A m u s s i u m p l e u r o - n e c t e s (see Text-fig. 1), and it is also extremely probable that the interlocking arrangement functions in a similar manner. He wrote: 'the ordinary filaments on either side of the principal filaments possess a ridge appearing in section as a spur turned towards the principal filaments. The principal filaments themselves possess lateral extensions, so that the whole system forms an accessory interlocking arrangement and may be looked upon as an interesting mechanical device by means of which the flapping extensions on the principal filaments fit into the groove formed by the spur. . . ., the principal filaments are extremely active and are responsible for what is described as the "flapping" movement.'[1]

The one or two filaments at the apex of the plica are larger than the rest of the ordinary filaments, except toward the lower region of the demibranch. The six or so filaments forming the plical crests appear to have longer frontal cilia than the others.

The P a l p s .—In the preserved state the folded surface of

[1] Setna examined P e c t e n m a x i m u s, Chlamys opercularis, and Chlamys tigerina. It has been found that a ridge also occurs on the filaments adjacent to the principal filaments in Chlamys distorta.
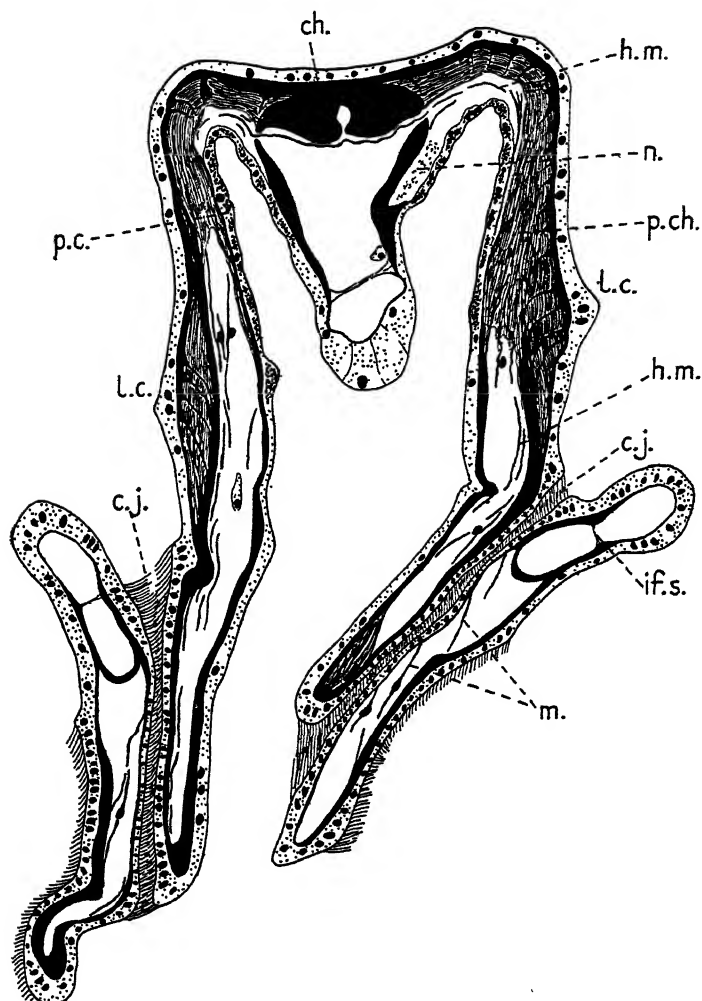
TEXT-FIG. 2.

Amussium pleuronectes. Transverse section of a principal and adjacent ordinary filaments (descending lamella) taken near the middle of the demibranch. *ch.*, darkly staining chitin; *c.j.*, ciliary junction; *h.m.*, horizontal muscles; *if.s.*, intrafilamentar septum; *l.c.*, position of lateral cilia; *m.*, muscle-fibres in spur of ordinary filament; *n.*, nerve?; *p.ch.*, pale staining chitin; *p.c.*, pigment cells. Alcohol fixation; Mallory's triple stain. ×456.

the almost rectangular palps is white, and the smooth surface dark brown. The inner and outer lips appear to be fused, or intricately interlaced over the mouth, which is thus hidden.

DISCUSSION.

It was considered of interest to examine any species of Amussiidae as this was a family which in 1888 was placed in the Pectinacea by Pelseneer (pp. 12, 13), was removed by Ridewood in 1903 (pp. 181, 185–6) to the Mytilacea, and was replaced in the Pectinacea by Pelseneer (1906, 1911).

Ridewood based his classification on the form of the gill, which he described as having 'flat lamellae and filaments undifferentiated' in the three species which he examined. These three species, Amussium dalli, Amussium meridionale, and Amussium lucidum are all deep-water forms. Amussium dalli Smith was found in 218 to 1,591 fathoms (Smith, 1885, pp. 308–9; Dall, 1885–6, pp. 209–10); Amussium meridionale Smith in 1,375 to 1,800 fathoms, and Amussium lucidum Jeffreys in 675 to 1,000 fathoms (Smith, 1885, pp. 316, 317). By ill chance he does not seem to have had the opportunity of examining Amussium pleuronectes, a shallow-water form, taken by the Challenger Expedition in 20 to 28 fathoms (Smith, p. 308) and by the Siboga Expedition in 18 to 82 metres (Dautzenberg and Bavay, 1912, p. 35).

Ridewood (1903, pp. 207–8) briefly described the gills of Amussium dalli, Amussium meridionale, and Amussium lucidum as follows: 'In the three species of Amussium examined the upper edges of the ascending lamellae are free from adjacent parts. The upper ends of the ascending filaments are united in series by ciliated discs, as also are the lower ends of the filaments along the ventral edge of the demibranch. There are no ciliated discs besides these two rows. There are no interlamellar junctions of any kind. The ascending lamellae of Amussium dalli reach nearly as high as the descending, but those of Amussium lucidum and Amussium meridionale only extend half-way up

the descending lamellae, or a little higher. An intrafilamentar septum is present, and the chitinous lining is of fairly uniform thickness.'

Pelseneer (1911, pp. 29, 97) who examined A m u s s i u m p l e u r o n e c t e s for the Siboga Report makes the general statement that the gills of A m u s s i u m are smooth. So that there should be no question of the correct identification of my specimens with plicate and heterorhabdic gills, Mr. R. Winckworth has courteously verified the name attached by the authorities of the Indian Museum.

The position of A m u s s i u m p l e u r o n e c t e s in the Pectinacea is supported, not only by the general anatomy, but by the detailed structure of the gills. The condition of the material renders it impossible to make any statement as to the form of the latero-frontal cilia—or indeed of any cilia—but the gill is so similar to that of P e c t e n that it is most probable that these will be found to be tenuous and small, that is micro-laterofrontal cilia (see Atkins, Part VII).

The Amussiidae then includes species with flat lamellae and at least one with plicate and heterorhabdic lamellae: it would seem unjustifiable to separate them because of this difference in the structure of the gills, placing those with flat lamellae in the Mytilacea and A m u s s i u m p l e u r o n e c t e s in the Pectinacea. Curiously enough, though Ridewood (1903, p. 181) stressed the difference between flat and homorhabdic, and plicate and heterorhabdic lamellae in the Eleutherorhabda, yet in the Synaptorhabda he (p. 161) considered that 'The differences presented by S o l e n and its immediate allies show that the plication of the lamellae and the differentiation of principal filaments are of not more than specific, or at most subgeneric, value'. In the Pectinidae also two types of gills occur; though most species have plicate and heterorhabdic lamellae, one at least, P e c t e n g r o e n l a n d i c u s, has homorhabdic lamellae (Haren-Noman, 1881–2, pp. 28–30.

Dakin (1928 a, pp. 358–9) has already expressed the opinion that the method of classification by the structure of the gill alone undoubtedly led Ridewood astray in the case of A m u s - s i u m, and he suggested that 'both on the ground of general

anatomy, as well as on the evidence from a study of the eye, Amussium be brought back to the Pectinidae'.

It is of interest that the three species from deep water should have simple, flat gills, with ciliated interfilamentar junctions restricted to the free lower edge of the demibranch and the upper ends of the ascending filaments. Plication increases the food-collecting surface of the gills, and it might have been expected that deep-water Amussiidae, living where phytoplankton is likely to be extremely scarce, would exhibit such plication. Actually they have flat gills, while the shallow-water species, Amussium pleuronectes, living where phytoplankton is probably comparatively rich, has highly plicate gills with markedly differentiated principal filaments. It may be that the simplicity of the gills of the deep-water species is to be explained by backwardness or retrogression:[1] the temperature at great depths would involve a low rate of metabolism. According to Dall (1885–6, p. 210) Amussium dalli is without palps; so it is possible that it takes in all particles that come to the gills, unless sorting occurs on the filaments as in certain bivalves (see Atkins, 1936, Part I).

Correlated with the difference in the structure of the gills of the deep-water species and Amussium pleuronectes there must be a considerable difference in the frontal currents on the filaments. In Amussium pleuronectes in all probability the gill currents will be found to be similar, if not identical, with those of species of Pecten and Chlamys with plicate and heterorhabdic gills; the deep-water species having simple, flat gills necessarily cannot have the same type. If the examination of the living gills of these forms was feasible it would be of extreme interest, and might very possibly reveal currents in opposite directions on the same gill filament as in the Arcidae, and on certain of the ordinary filaments of Pecten, Chlamys, and others (see Atkins, 1936, 1937).

[1] In the genus Donax Rice (1897, see Ridewood, 1903, p. 162) regarded the simplicity of the flat forms as secondary, and derived by retrogression from the plicate condition.

Summary.

The gills of Amussium pleuronectes L., a species from shallow water, were examined and the lamellae found to be plicate and heterorhabdic, thus differing in structure from those of the deep-water species, Amussium dalli, Amussium meridionale, and Amussium lucidum, which Ridewood found to have flat and homorhabdic lamellae. The gills of Amussium pleuronectes closely agree with those of the Pectinidae also possessing plicate and heterorhabdic lamellae.

Ridewood's classification of the Amussiidae with the Mytilacea cannot be upheld; the position of this family is with the Pectinacea as in Pelseneer's classifications of 1888, 1906, and 1911.

On the Ciliary Mechanisms and Interrelationship of Lamellibranchs.

PART VI: The Pattern of the Lateral Ciliated Cells of the Gill Filaments of the Lamellibranchia.

By

Daphne Atkins, B.Sc.

Marine Biological Laboratory, Plymouth.

With 6 Text-figures.

CONTENTS

INTRODUCTION.

THE lateral ciliated cells of the gills of Lamellibranchs are arranged in a definite manner, which appears to be constant at least for the species. Though there may be considerable variation in the length of the cells in different parts of the same filament (see Text-fig. 5 G), yet the arrangement, or pattern, is the same. In some instances the same general pattern is characteristic of a genus, and even of a family or larger group. The cells from which the lateral cilia arise are rhomboidal, frequently almost rectangular, at the surface, and elongated in the direction of length of the filaments; in some species, however, there is little difference between the length and breadth of certain of the cells, as in Pteria hirundo (Text-fig. 2 F), Phacoides (= Lucina) borealis (Text-fig. 4 B), and Macoma balthica (Text-fig. 5 A), and practically no difference in Teredo navalis (Text-fig. 4 A). In some

patterns the transverse cell-walls of the several rows are more or less in line; in others they are distinctly irregular.

Engelmann in 1880 gave figures of the arrangement of these cells in Anodonta, Cyclas, and Ostrea. He records (p. 513) that 'Form und Grösse der Seitenzellen sind weder bei den verschiedenen Arten, noch auch bei den verschiedenen Zellreihen derselben Art die gleichen': he, however, apparently examined few bivalves. This author (1880, pp. 513–14) was perhaps the first to observe the arrangement of the basal granules of the cilia in oblique rows, which he stated were orientated obliquely at an angle of 45° across the surface of the cell in Anodonta, Unio, Cyclas, Mytilus, and Ostrea. According to Saguchi (1917, p. 221) the rows are transverse to the length of the cell in Anodonta; while Lucas (1932 a, p. 271) found the angle of inclination to average 35° in Modiolus demissus.

Oblique arrangement of the basal granules of the cilia was observed in the lateral cells of all the species of Lamellibranchs examined, but the exact angle was not determined and the rows therefore are not shown in the figures. The granules stain well with Heidenhain's iron haematoxylin, and indicate the shape and arrangement of the cells clearly in sections parallel to the surface. Slight obliquity of the sections, however, may have resulted in the cells being figured slightly wider or narrower than they actually are. The pattern of the lateral ciliated cells in the majority of the bivalves was determined in this way, and mostly checked either from transverse sections or from the living filaments. The patterns are drawn somewhat diagrammatically in that the cells outlines have been straightened. In all the figures the frontal edge of the lateral ciliated tract is on the right.

The curiously narrow rows of basal granules, which, when present, generally occur on the outer sides (frontal and abfrontal) of the tract (Text-figs. 2, 3), appear to be actually on separate cell rows, at least where they have been checked in the living filaments (e.g. Arca tetragona, Glycymeris glycymeris, Heteranomia squamula, Monia squama, Ostrea, Mytilus edulis). It was found impossible

accurately to distinguish breaks in these rows, indicating limits of cells, and in the figures they are shown as continuous lines. The significance of these exceedingly narrow rows of cilia is unknown; it would seem improbable that their chief value can be that of current producers. The lateral cells increase considerably in size basally, as can be seen in transverse sections, and as is indicated by the fact that in figures showing the disposition of the nuclei (Text-figs. 4 A (a); 6 A (a)) these cover a greater area than the cells at the surface.

THE PATTERN OF THE LATERAL CILIATED CELLS.

I. Protobranchia.

In the Protobranchs, Nucula radiata Hanley (Nuculidae), Nuculana (= Leda) minuta (Müller) (Nuculanidae), and Solenomya togata (Solenomyidae), the lateral ciliated tract is six, seven, or eight cells wide; the ends of the cells being pointed and interdigitating, except those of the outer row on the abfrontal side, which are more or less rectangular and placed end to end (Text-fig. 1). The figure of the lateral cells of Solenomya togata (Text-fig. 1 c) has been composed from two or three sections, but it shows that the cells are longer than in Nucula radiata (Text-fig. 1 A) and Nuculana minuta (Text-fig. 1 B), and that there is not only a tendency for the cells to assume the rectangular shape, but for them to be arranged in definite rows. The somewhat more indefinite, or more primitive, arrangement of the lateral cells would seem to be that occurring in Nucula and Nuculana.

Although tracts of six cells wide are met with among the higher Lamellibranchs they do not attain such a width as in the two Protobranchs Nucula and Solenomya, namely about 26μ in Nucula radiata and about 30μ in Solenomya togata. In Nuculana minuta the tract is about six cells wide, and the total width about 18 to 22μ. The greatest width observed in a higher Lamellibranch was about 19μ in Phacoides (= Lucina) borealis. Measurements mentioned in this note were made on sections, and are therefore probably considerably less than they would be on living tissue.
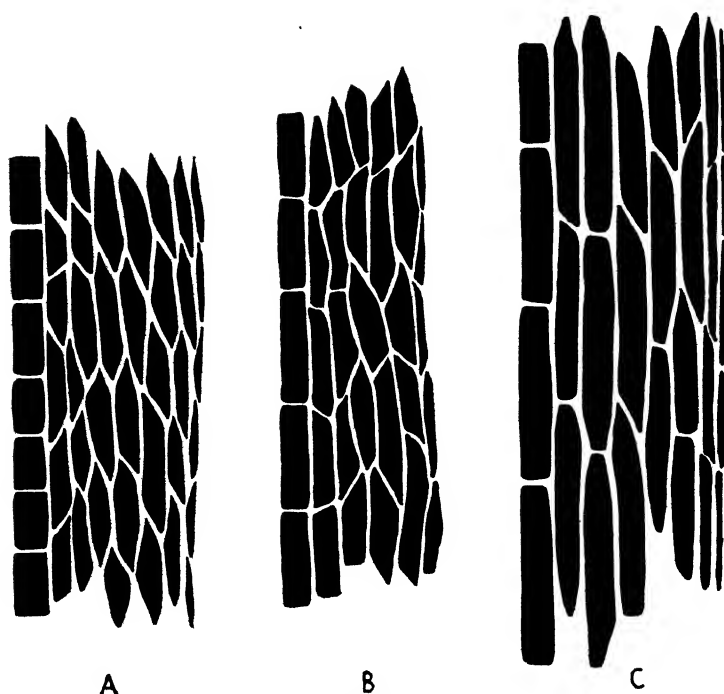
TEXT-FIG. 1.

Lateral ciliated cell patterns in Protobranchia. A, Nucula
radiata (Nuculidae); B, Nuculana minuta (Nuculanidae);
c, Solenomya togata (Solenomyidae). The frontal edge of
the tract is on the right of the figure.   × 980.

II. HIGHER LAMELLIBRANCHIA.

A. Group Possessing Micro-latero-frontal Cilia.

In the group of Lamellibranchs having micro-latero-frontal
cilia (see Atkins, Part VII) the pattern of the lateral ciliated
cells is distinctly different in different families and even in
different genera, for instance among the Pteriidae.

Arcidae.—In the Arcidae practically the same arrangement
of the six cell rows is found in Arca tetragona Poli and
Glycymeris glycymeris (L.) (Text-fig. 2 A and B), though
the cells are somewhat longer in the former.
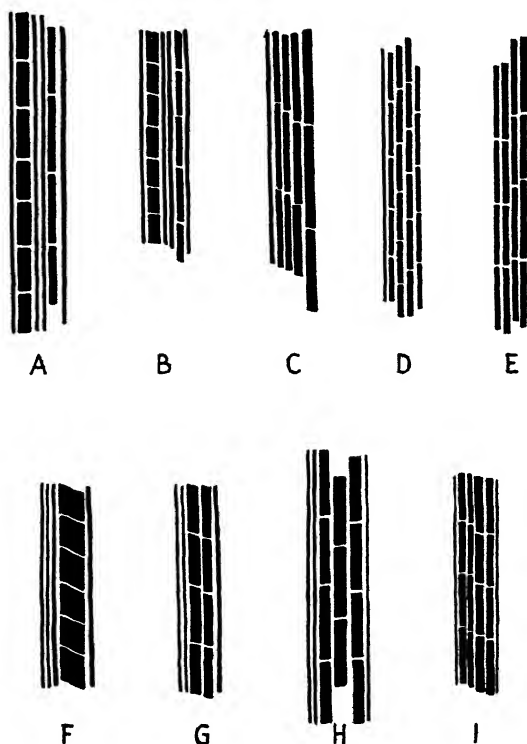
TEXT-FIG. 2.

Lateral ciliated cell patterns in the group of Lamellibranchs having
micro-latero-frontal cilia. A, Arca tetragona; B, Glycy-
meris glycymeris (Arcidae); C, Anomia ephippium
(Anomiidae); D, Pecten maximus (Pectinidae); E, Lima
hians (Limidae); F, Pteria hirundo (Pteriidae); G, Malleus
albus (Pteriidae); H, Pinna fragilis (Pinnidae); I, Ostrea
edulis (Ostreidae). The frontal edge of the tract is on the right
of the figure. It was found impossible accurately to determine the
cell limits of the very narrow rows, and these are therefore shown
as continuous. ×980.

Anomiidae.—In the Anomiidae a pattern of five-cell rows
is common to the three genera, Anomia (Text-fig. 2 c),
Heteranomia and Monia, though no doubt slight varia-
tions occur in the different genera and species.

Pectinacea.—In Pecten maximus (L.) (Pectinidae)

the narrow lateral ciliated tract, only about 5μ wide, is composed of five rows of cells, the outer on the abfrontal side being exceedingly narrow (Text-fig. 2 D). Five rows of cells also occur in Spondylus gaederopus (Spondylidae). In Lima hians (Gmelin) (Limidae) there appear to be but four rows of narrow cells (Text-fig. 2 E), and the total width little more than 4μ.

Pteriacea.—In the Pteriacea a striking pattern of a row of wide cells with four rows of very narrow ones, one on the frontal side and three on the abfrontal side, is found in Pteria hirundo (L.) (Text-fig. 2 F), Pinctada vulgaris, Pinctada margaritifera (Pteriidae), Isognomon alata (Isognomonidae), and in Vulsella sp. (Vulsellidae), while Malleus albus Lamarck (Pteriidae) has two rows of medium width and three very narrow rows (Text-fig. 2 G). The genus Malleus is not as old as the other genera mentioned, being unknown fossil.

Pinna fragilis Pennant (Pinnidae) has six rows of cells, three of median width and three very narrow (Text-fig. 2 H). In the Ostreidae, Ostrea edulis L. (Text-fig. 2 I), Ostrea virginica Gmelin and Ostrea angulata (Lamarck) have a common pattern of six rows of cells, four of median width and two very narrow, one on the frontal and one on the abfrontal side.

B. Group Possessing Eu-latero-frontal Cilia.

Outside the group of Lamellibranchs with micro-laterofrontal cilia the usual number of rows of lateral ciliated cells appears to be four, except in the Mytilidae (Mytilus edulis L., Modiolus modiolus (L.), Modiolus adriaticus Lamarck, Musculus (= Modiolaria) marmoratus (Forbes)) which have six rows, the two outer being exceedingly narrow (Text-fig. 3). In Musculus marmoratus, so far as can be judged from entire filaments preserved in formalin,[1] the fourth row from the frontal side is very narrow.

[1] In entire unstained filaments of formalin and alcohol preserved material the cell outlines are frequently observable, but in Bouin and Bouin-Duboscq preserved material they are not.

The majority of Eulamellibranchs investigated possess the same type of pattern (see Text-figs. 4, 5) of one row of broad cells, with a row of narrow ones to the frontal side, and two rows of narrow cells to the abfrontal side, of which the outer is sometimes the wider, for instance in Ensis, Solecurtus, Cultellus, Barnea parva, and Hiatella rugosa.
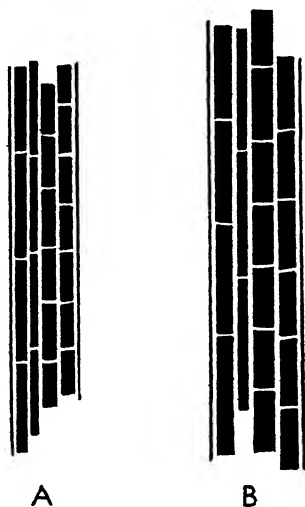
TEXT-FIG. 3.

Lateral ciliated cell pattern in the Mytilidae. A, Mytilus edulis; B, Modiolus modiolus. The frontal edge of the tract is on the right of the figure. It was found impossible accurately to determine the cell limits of the very narrow rows, and these are therefore shown as continuous.   × 980.

This general type of arrangement of the lateral ciliated cells was figured for Cyclas (= Sphaerium) cornea by Engelmann (1880, Pl. V, fig. 4) and by Wallengren (1905, fig. E, p. 45) in Mya. Two main variations exist; one in which the cells of the principal row are as wide, or almost as wide, as long (e.g. Teredo navalis, Text-fig. 4 A; Phacoides borealis, Text-fig. 4 B; Macoma balthica, Text-fig. 5 A); and the other in which they are distinctly longer than wide (e.g. Ensis siliqua, Text-fig. 5 F; Solecurtus;

Lutraria lutraria, Text-fig. 5 G; Sphaerium cor-
neum; Tellina tenuis, Tellina crassa, Text-fig. 5
B and C; Aloidis gibba, Text-fig. 5 E; Venus casina,
Text-fig. 5 D). No doubt there are specific, as well as generic
and family differences in the average width and length of the

A   A(a)   B
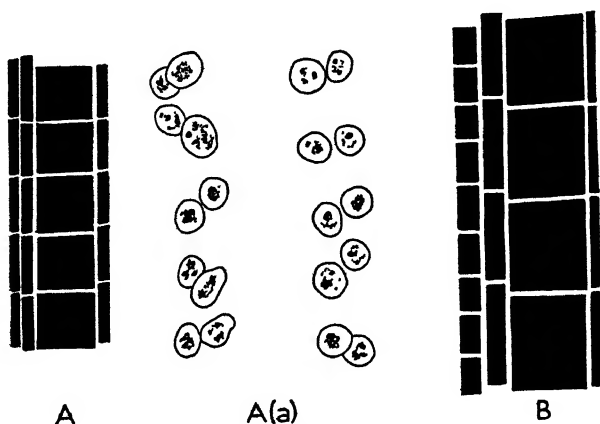
TEXT-FIG. 4.

Lateral ciliated cell pattern in two Eulamellibranchs. A, Teredo
navalis (Teredinidae); B, Phacoides borealis (Lucinidae);
A (a), arrangement of nuclei of lateral cells of Teredo navalis.
The frontal edge of the tract is on the right of the figure. ×980.

cells of the various rows; and, as previously mentioned, there
may be considerable differences in length of the cells in different
parts of the same filament. Text-fig. 5 G (a), shows lateral
ciliated cells from about the middle of an ascending filament
of Lutraria lutraria, and Text-fig. 5 G (b), from near the
dorsal end of the same filament. It is interesting that in the

crassa (Tellinidae); D, Venus casina (Veneridae); E,
Aloidis gibba (Erodonidae); F, Ensis siliqua (Solenidae);
G, Lutraria lutraria (Lutrariidae); G (a), from about midway
along the ascending filament of the outer demibranch; G (b), from
near the dorsal end of the same filament; H, Lyonsia nor-
wegica (Lyonsiidae). The frontal edge of the tract is on the
right of the figure. The figure of Macoma is drawn from the
living filament. A × 573⅓; B–H × 980.
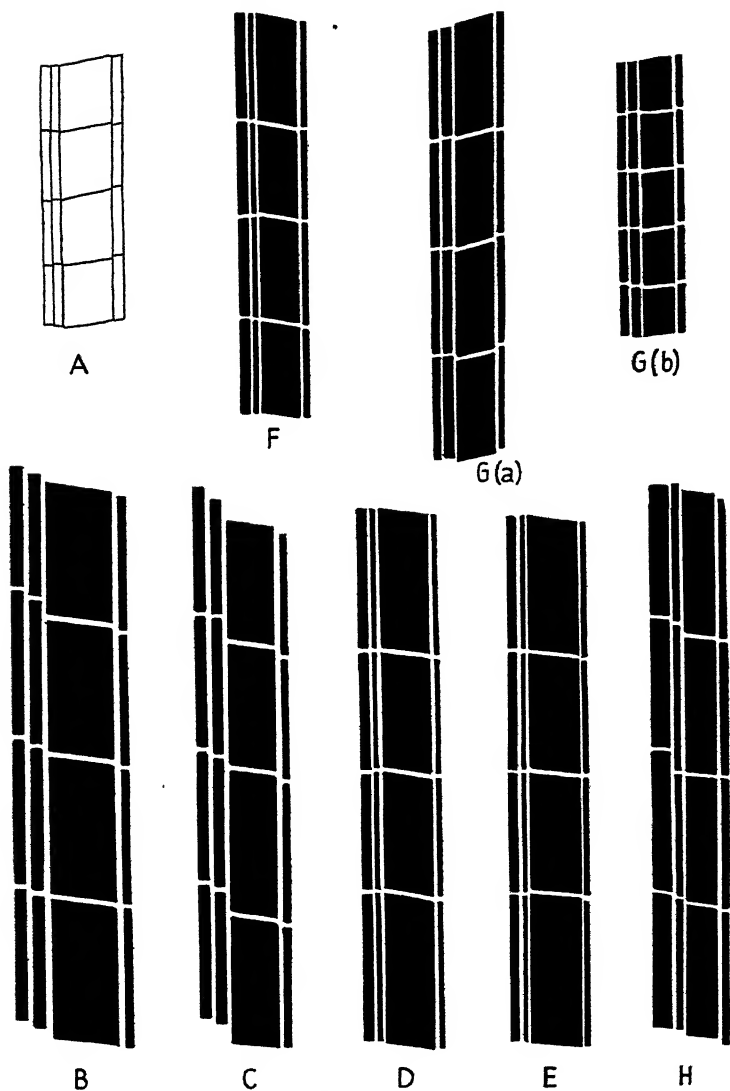
TEXT-FIG. 5.

Lateral ciliated cell pattern in some marine Eulamellibranchs. A,
Macoma balthica; B, Tellina tenuis; C, Tellina

Tellinidae, Macoma (Text-fig. ·5 A) has one variety, and Tellina (Text-fig. 5 B, c) the other.

In Lyonsia norwegica the main row of cells is not so noticeably broad as in the majority of the species, while the outer row on the abfrontal side is proportionately broader (Text-fig. 5 H).

Though the number of cell rows is constant the total width of the tract varies much in different bivalves, being especially broad in Phacoides borealis (ca. 19μ) and in Scrobicularia plana (16–18μ). The larger size of the cells of Tellina tenuis in Text-fig. 5 B than of Tellina crassa in Text-fig. 5 c is possibly due to the fact that the specimen of Tellina tenuis from which the gills were taken was a large one, 4·3 cm. long, and that of Tellina crassa a young one only 1 cm. long; there is perhaps an increase in the size of the cells with age.

Bivalves having a lateral ciliated cell pattern of a row of large cells, with one row of narrow ones to the frontal side, and two to the abfrontal side, are the following:

Dreissensiidae: Dreissensia polymorpha (Pallas).

Cyprinidae: Cyprina islandica (L.).

Lucinidae: Phacoides (= Lucina) borealis (L.), (Text-fig. 4 B).

Montacutidae: Mysella bidentata (Montagu).

Erycinidae: Kellia suborbicularis (Montagu).

Sphaeriidae: Sphaerium (= Cyclas) corneum (L.) (see Engelmann, 1880).

Tellinidae: Tellina tenuis da Costa (Text-fig. 5 B): Tellina crassa Pennant (Text-fig. 5, c); Macoma balthica (L.) (Text-fig. 5 A).

Semelidae: Scrobicularia plana (da Costa) (= piperata).

Asaphidae: Gari fervensis (Gmelin) (= ferroensis).

Donacidae: Donax vittatus (da Costa).

Mactridae: Spisula elliptica (Brown).

Lutrariidae: Lutraria lutraria (L.) (= elliptica) (Text-fig. 5 G).

Myidae: Mya (see Wallengren, 1905).

Veneridae: Venus casina L. (Text-fig. 5 D), Venus
striatula (da Costa) (= gallina), Paphia decus-
sata (L.).
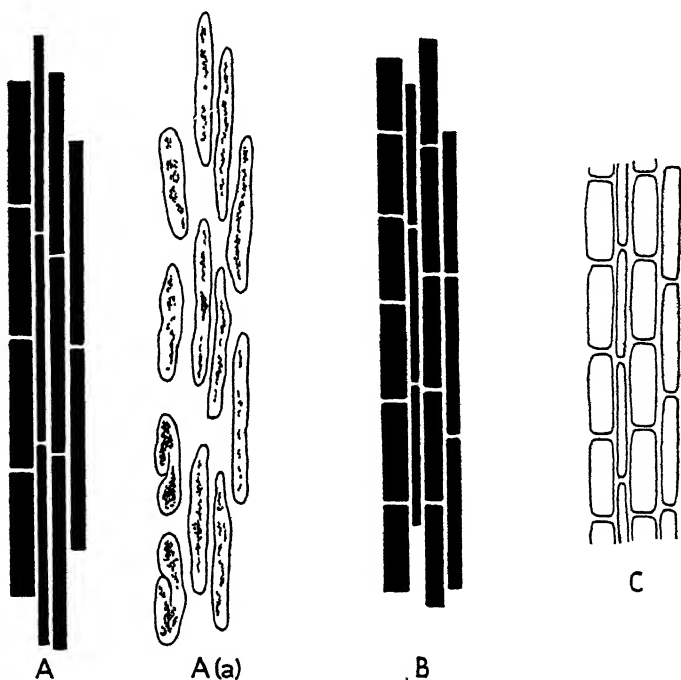
TEXT-FIG. 6.

Lateral ciliated cell pattern in A, Astarte sulcata (Astartidae);
B, Aetheria elliptica (Aetheriidae); C, Anodonta
(Unionidae), after Engelmann, 1880. A (a), arrangement of nuclei
of lateral cells of Astarte sulcata. These are not the actual
nuclei of the cells figured in A. The frontal edge of the tract is on
the right of the figure. A and B × 980.

Cardiidae: Cardium crassum Gmelin (= norvegi-
cum).

Erodonidae: Aloidis (= Corbula) gibba (Olivi) (Text-
fig. 5 E).

Solenidae: Ensis siliqua (L.) (Text-fig. 5 F), Solen mar-
ginatus Montagu, Cultellus pellucidus (Pennant).

Solecurtidae: S o l e c u r t u s s c o p u l a (Turton) (= c a n d i-
    d u s), S o l e c u r t u s c h a m a s o l e n (da Costa) (= a n t i-
    quatus).
Hiatellidae: H i a t e l l a g a l l i c a n a (Lamarck) (= r u g o s a).
Pholadidae: B a r n e a p a r v a (Pennant), X y l o p h a g a
    d o r s a l i s Turton.
Teredinidae: T e r e d o n a v a l i s L. (Text-fig. 4 A).
Lyonsiidae: L y o n s i a n o r w e g i c a (Gmelin) (Text-fig. 5 H)
    In A s t a r t e s u l c a t a (Text-fig. 6 A) the four rows of lateral
cells form a very different type of pattern from that just
described. The narrow cells are extremely long, those of some
rows reaching 28μ, with nuclei about 23μ long. It is interesting
to contrast these cells and nuclei with those of T e r e d o
n a v a l i s (Text-fig. 4 A).
    There is not a great deal of difference in the patterns of the
lateral ciliated cells of A e t h e r i a e l l i p t i c a (Aetheriidae)
(Text-fig. 6 B) and of A n o d o n t a (Unionidae) (Text-fig. 6 C),
two of the Naiadacea. In A n o d o n t a, judging from Engel-
mann's figure (1880, Pl. V, fig. 2), reproduced in part as Text-
fig. 6 C, the second row from the frontal side is somewhat wider
than the outer on the abfrontal side, while in A e t h e r i a
(Text-fig. 6 B) the reverse obtains. The arrangement of the
elongated cells of A e t h e r i a e l l i p t i c a is surprisingly like
that of the marine A s t a r t e s u l c a t a, but is almost certainly
due to accidental convergence.
    T r i g o n i a m a r g a r i t a c e a (Trigoniidae) has four cell rows
of more or less the same width, so far as can be determined from
alcohol preserved material: the cells are elongated.

DISCUSSION.

It is interesting that in the most primitive order of Lamelli-
branchs, the Protobranchia, the cells of the lateral ciliated
epithelium have for the most part no definite shape or arrange-
ment, their ends being pointed and interdigitating. A single
row of elongated, almost rectangular cells placed end to end is
present on the abfrontal side of the tract, though in S o l e n o-
m y a t o g a t a—in which the gills are larger and more important
as food collectors than in N u c u l a and N u c u l a n a m i n u t a,

which are chiefly deposit feeders—there is a tendency for other cells to assume this shape and arrangement. In the higher Lamellibranchs there is an orderly arrangement of the cells, all being more or less rhomboidal in shape at the surface and arranged end to end in definite rows. Possibly this orderly arrangement of the lateral cells allows of the more efficient working of the ciliary mechanism, than does that of the Protobranchs. It is noteworthy that in all the patterns described some, or all, of the cells are elongated in the direction of the length of the filaments, and still more important in the direction of travel of the metachronal wave.

In the Protobranchs the lateral ciliated tracts are wide, 18 to 22μ in Nuculana minuta, about 26μ in Nucula radiata, and about 30μ in Solenomya togata. Wide tracts are probably primitive, for they have been depicted in transverse sections of the filaments and leaflets of certain Gastropods (Pelseneer, 1891, Pl. XXIII; Woodward, 1901, Pl. 14, fig. 18; Orton, 1912, fig. 5; Crofts, 1929, Text-fig. 10, p. 48). In the higher Lamellibranchs not only are the lateral ciliated cells arranged in an orderly manner, but there is a tendency to reduction in width of the tract, and this is especially evident in the group possessing micro-latero-frontal cilia: in Lima hians the tract is little more than 4μ wide. The greatest width observed in a higher Lamellibranch was about 19μ in the Eulamellibranch Phacoides borealis, while Scrobicularia plana comes very near this with a width of 16 to 18μ.

The variation in the pattern of the lateral ciliated cells in the various families of the group having micro-latero-frontal cilia (see Atkins, Part VII), is in marked contrast with the constancy of the general type of pattern found in the Eulamellibranchs, with the exception of Astarte, Anodonta, and Aetheria. The lateral ciliated tracts in the former group are altogether finer than in the latter, being distinctly narrower and composed of smaller cells—the width of the tract and the size of the component cells does not appear to be primarily dependent on the size of the filament. I do not think that it follows, however, that the inhalent current necessarily is less strong in consequence; in fact the current set up by the lateral cilia in

the Anomiidae seems particularly violent, owing probably to the rapidity of their beat.

The extraordinary constancy of the general pattern found in members of twenty-two families of Eulamellibranchs is curious, and seems to point to their close relationship. Some ciliary structures of the gills are obviously adaptive, being correlated with certain habits and habitats (see Atkins, 1937), but the lateral cilia of the gill filaments are found in all Lamellibranchs and many Gastropods independently of these. Though the lateral cilia are such important members of the ciliary feeding complex, the exact and detailed arrangement of the cells bearing them would seem to have doubtful utility, and where the same pattern is found in different families would seem to indicate their close relationship. The disposition of the lateral ciliated cells is evidently a stable character in Eulamellibranchs, but a variable one in the group of Lamellibranchs with microlatero-frontal cilia.

Eulamellibranchs with a similar arrangement of the lateral cells, namely a row of large cells, with one row of narrow ones to the frontal side, and two to the abfrontal side, are found both in Douvillé's (1912 a, p. 466) 'normal' and 'burrowing' branches. To discover whether the same pattern occurs in all the families of these two lines, or is even common to all the members of a family, will need the examination of many more species; in most families it has been possible to examine only one member.

SUMMARY.

The pattern of the lateral ciliated cells of the gill filaments has been examined in a number of Lamellibranchs and figures given. In the Protobranchia the lateral ciliated cells, except for a row on the abfrontal side, have no definite shape or arrangement; in the higher Lamellibranchs there is an orderly arrangement of the approximately rhomboidal cells in rows. The arrangement of the cells in any species appears to be constant. In the group possessing micro-latero-frontal cilia the variation in the pattern of the lateral ciliated cells in the various families is in marked contrast with the constancy of the general type of pattern found in the majority of the Eulamellibranchs.

On the Ciliary Mechanisms and Interrelationships of Lamellibranchs.

PART VII: Latero-frontal Cilia of the Gill Filaments and their Phylogenetic Value.

By

Daphne Atkins, B.Sc.

Marine Biological Laboratory, Plymouth.

With Plate 29, and 12 Text-figures.

Contents.

INTRODUCTION.

THE value of ciliary structures in the classification of the Lamellibranchia must depend largely on a knowledge of their form and of their distribution in the class of animals under consideration, and to some extent of their function. Some ciliary mechanisms, such as tracts of cirrus-like frontal cilia on the gills are obviously adaptive, being correlated with certain habitats and modes of life, namely sand dwelling and rock and wood boring (Atkins, 1937). The presence of fan-shaped groups of long cilia—guarding cilia—along the marginal food grooves of the gills also seems to be adaptive, being correlated with a certain amount of mud or silt in the soil (Atkins, 1937). Such adaptive ciliary structures may have little or no taxonomic value.[1] Those instanced are not restricted to closely related forms, and in a family one member may possess and one lack them.

Certain tracts of cilia[2] forming an essential part of the ciliary

[1] It is not implied that because a character is useful and adaptive it cannot serve in classification, but that among the Lamellibranchia, which appear to be very adaptable, adaptive characters must be used with caution.

[2] The terms frontal, latero-frontal, and lateral, which have been applied to the three tracts of food-collecting cilia on the gill filaments (see Ridewood, 1903, p. 163) are descriptive of the position these cilia occupy in the great majority of Lamellibranchs, but they have come to denote cilia with certain characteristics, especially as to function, and are therefore applied in instances where they are not descriptive of position, at least in preserved material, and in some instances most probably on the living gill. For example, the frontal cilia may extend well round on to the lateral faces of the filaments, and the latero-frontal cilia may be clearly lateral in position as in Nuculana minuta (Text-fig. 8) and Trigonia margaritacea (Text-fig. 1 c).

feeding complex, though independent of habit and habitat, are so universally present on the gills, with little or no modification of their form, that these again are useless in classification. Such are the lateral cilia of the gills, which create the inhalent current, found in all Protobranchia, Filibranchia, Pseudolamellibranchia, Eulamellibranchia, and even on the few filaments forming the branchial sieve in Poromya among Septibranchia, as well as in certain aspidobranch and pectinibranch Gastropods: analogous tracts of cilia occur in several groups of ciliary feeders outside the Mollusca. The mere presence of such cilia therefore has no classificatory value, but the arrangement or pattern of the cells bearing them may be of generic, and family, and possibly of wider, value; for instance, the same type of cell-pattern is characteristic of a large number of eulamellibranch families (Atkins, 1938 a).

The frontal, food conveying, cilia of the gills are also an essential element in the feeding complex, and too widely present to be of value in classification. Modification of these cilia seems to be largely in relation to habitat (Atkins, 1937). But the modification in which adjacent antagonistic tracts of frontal cilia on the same gill filament occur on all lamellae, is found chiefly in one group of Lamellibranchs, 'the Aviculidae and their allies', but is not entirely restricted to this group—it is found also in the Solenidae (Atkins, 1936)—and is not found in all its members, for instance not in Pinna (Atkins, 1937).

The latero-frontal cilia of the gills form an additional part of the ciliary feeding complex, but, apparently, are not absolutely essential, as are the lateral (current producing) and frontal (food conveying) cilia, for they have been described only in Lamellibranchs, not in Gastropods; nor have analogous cilia been described in ciliary feeders outside the Mollusca. They are straining cilia, preventing the unimpeded passage of particles between the gill filaments to the supra-branchial chamber. They thus contribute to the efficiency of the method of feeding, preventing the escape and wastage of perhaps desirable food particles, but would not seem to be indispensable.

Two chief types of latero-frontal tracts have been found to exist, the one efficient, composed of a row of large, with also

a second row of small latero-frontal cilia, on each side of the
frontal surface; the other apparently rather inefficient, composed
of one row only of tenuous and small latero-frontal cilia on each
side. The types are unconnected with habits or habitats. The
distribution of the second type among Lamellibranchs is not
irregular, but is distinctive of a particular group. It may seem
ridiculous to consider that the composition of a certain tract
of gill cilia can be of value in determining genetic affinity, but
the fact remains, that without any preconceived ideas, and
setting out merely with the intention of spending odd minutes
noting what proportion of Lamellibranchs had large latero-
frontal cilia, and what proportion had small ones—or, as was
at first thought, were without them—it was gradually realized
that the occurrence of only small latero-frontal cilia is character-
istic of members of a group 'the Aviculidae and their allies' or
the 'sedentary' branch of Lamellibranchs established by palae-
ontologists (Jackson, 1890; Douvillé, 1912a) mainly, or entirely,
on shell characters. One desideratum of a character for use in
determining phylogeny, namely conservatism, seems to be
possessed by the latero-frontal ciliated tracts, for, so far as the
present work has gone, there appears to be little variation within
the two types, and they therefore afford an important and
reliable character for purposes of broad classification.

In addition to the acknowledgements already made in Part I
of the series, I wish to thank Professor A. Morley Davies for
criticizing the present paper from the palaeontological view-
point, and to renew my thanks to Professor J. H. Orton for
most helpful general criticism of this paper in particular. Pro-
fessor E. S. Goodrich has not only edited it, but has made
suggestions for which I am much indebted to him.

MATERIAL AND METHODS.

The great bulk of the material used for this, as for the other
papers of the series, was brought in by S. S. Salpa of the Marine
Biological Association of Plymouth. Living specimens of
Nuculana minuta and Musculus discors were ob-
tained from Millport Marine Station by purchase, as were also
living specimens of Petricola pholadiformis from Whit-

stable, and Bouin-Duboscq-preserved specimens of Pteria hirundo, Spondylus gaederopus, Anomia ephippium, Solenomya togata, and Nuculana pella from the Zoological Station of Naples.

My thanks are due to a number of zoologists who have most kindly furnished me with valuable material: to Dr. B. Prashad of the Indian Museum, Calcutta, for alcohol-preserved specimens of Amussium pleuronectes, Pinctada vulgaris, Pinctada margaritifera, Malleus albus, Isognomon isognomon var. canina, and Arca (Scaphula) celox; to Mr. G. C. Robson of the British Museum for fragments of the gills of Trigonia margaritacea, Placuna placenta (?), Vulsella sp., Isognomon alata, Aetheria elliptica, and Mülleria dalli; to Mr. A. G. Lowndes for living specimens of Anodonta anatina and Sphaerium corneum; to Mr. C. Oldham for living Dreissensia polymorpha; to Mr. G. A. Steven for a formalin-preserved specimen of Chlamys vitrea; and to Professor C. M. Yonge for Bouin-preserved specimens of Spondylus sp. from the Great Barrier Reef.

I am indebted to Professor J. H. Orton for the loan of Sir W. A. Herdman's slides of the gills of Pinctada vulgaris and Placuna placenta from Liverpool University, and to Mr. H. H. Bloomer and Mr. G. C. Robson for the loan of slides of the gills of Mutela bourguignati.

The form of the latero-frontal cilia of the gills is discoverable most rapidly and reliably from the examination of living material, and this was done whenever possible. When preserved material only was available, entire filaments were examined unstained in alcohol, or formalin, with a drop or two of glycerine added, and the results obtained verified by the examination of stained sections.

Material was fixed in Bouin-Duboscq's fluid (see Atkins, 1937 b, p. 424). Sections were cut 5μ or 6μ thick. The stains chiefly employed were Heidenhain's iron haematoxylin, either alone or counter stained with acid fuchsin, and Mallory's triple stain.

All drawings, except Text-figure 2 c, were made with the aid of a camera lucida.

A. THE LATERO-FRONTAL CILIATED TRACTS
OF THE GILL FILAMENTS.

THE OCCURRENCE OF LARGE OR EU-LATERO-FRONTAL CILIA, TOGETHER WITH SUBSIDIARY OR PRO-LATERO-FRONTAL CILIA.

It has been known for a number of years that some Lamellibranchs have large latero-frontal cilia (eu-l-f.c., Text-fig. 1), or cilia of the corner cells, on the gills (Peck, 1877; Engelmann, 1880; Janssens, 1893, &c.), though some of the earlier workers did not discern the direction of their effective beat or discover their function. These cilia are large, cirrus-like, and impossible to overlook, and, as described by Orton (1914), have 'the appearance of flexible combs working along the sides of the filaments'. They are present on each side of the tract of frontal cilia and 'stand out from the sides of the filaments, forming a sort of grating between them, and lash relatively slowly across the length and towards the middle of the frontal face of the filament' (Orton, 1912). The latero-frontal cilia are straining cilia, and throw particles on to the frontal face of the filament whence they are transported by the frontal cilia.

The structure and movement of these large cilia have been investigated by numerous workers (Engelmann, 1880; Janssens, 1893; Wallengren, 1905, I; Gray, 1922, 1928; Carter, 1924; Grave and Schmitt, 1925; Bhatia, 1926).

Carter's (1924) account is the most detailed. He investigated

Transverse sections of filaments of marine Lamellibranchs having eu-latero-frontal cilia. A, Modiolus modiolus; B, Kellia suborbicularis; C, Trigonia margaritacea. Fig. 1 C was composed from several sections. *abf.c.*, abfrontal cilia; *c.d.*, ciliated disc; *c.f.c.*, coarse frontal cilia; *c.r.*, calcified rods; *eu-l-f.c.*, eu-latero-frontal cilium; *f.c.*, frontal cilia; *f.c.l.*, long frontal cilia borne on three or four rows of long cells; *f.f.c.*, fine frontal cilia; *l.c.*, lateral cilia; *m.g.*, mucous gland; *pro-l-f.c.*, pro-latero-frontal cilium; *t.m.*, transverse muscle-fibre. The chitinous skeleton is shown in black, except in Trigonia margaritacea where it is shaded and the calcified rods shown in black. A and B Bouin-Duboscq's fixative; C, alcohol fixation; A–C, iron haematoxylin and acid fuchsin. × 735. For this, and other figures of gill filaments, sections have been chosen which were as free as possible of mucous glands, as these interfere with the orderly arrangement of the ciliated cells.
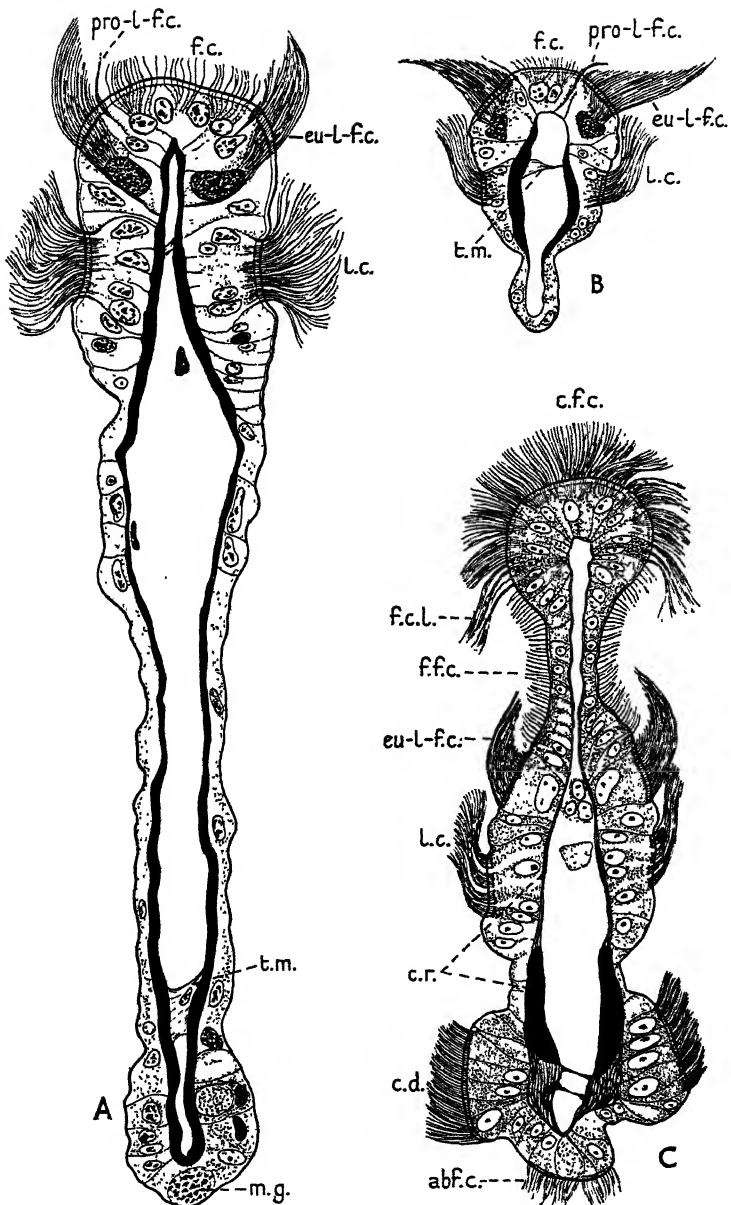
TEXT-FIG. 1.

[For description see previous page.]

these large, complex cilia by means of the micro-dissection needle and found that though they appear homogeneous when living, they are composed of a series of triangular plates (10 to 15 in Mytilus galloprovincialis) set one behind the other in the plane of the beat, with the shorter in front (Text-fig. 2 c, a, b, p. 355), that is on the side which is directed forward in the effective stroke (Text-fig. 2 c, c). These plates together form a blade-shaped compound cilium with the flat side of the blade in the plane of the beat (Text-fig. 2 c). When the cilium dies, or on fixation, the plates forming the cilium break up, and there is left a double row of fibres (individual cilia) formed by their edges. Below each fibre lies a basal granule; there is therefore a double row of granules below each latero-frontal cilium.

A ciliary structure of this complexity cannot rightly be called a cilium, but as the term latero-frontal cilium is well established in the literature it is retained here.

Gray (1928, p. 18) has described how the latero-frontal cilium is perfectly straight when at rest and that 'movement occurs by a flexure which begins at the tip and passes down to the base thereby bending the cilium into a hook-shaped structure', while 'during recovery the process is reversed for the cilium straightens from the base to the tip' (Text-fig. 2 c, c.).

In Lamellibranchs with large latero-frontal cilia there is, in addition, along the frontal side of these, a row of cilia which may be considered either as specialized frontals, or, perhaps more correctly, as a second row of small or subsidiary latero-frontal cilia (*pro-l-f.c.*, Text-figs. 1, 2 A). They are difficult to observe, being over-shadowed by the large ones, but it has been determined that they are in a single row, and that the beat is toward the frontal surface—as is that of the latero-frontal cilia—though somewhat obliquely in the direction of beat of the frontal cilia. They are more closely set than the large cilia, and are borne a number on a cell. The cells which bear them are narrow and elongated in the direction of length of the filament. In Ensis siliqua one cell covers the same length as do about eight large latero-frontal cells (Text-fig. 2 B); in Anodonta as six or seven, and in Cyclas cornea (= Sphaerium corneum) as about ten (see Engelmann, 1880, Pl. V, figs. 2, 4). The number of

these subsidiary latero-frontal cilia to a cell could not be determined, but, as they are more closely set than the large ones, there would be more than eight, six or seven, and ten respectively to a cell, and possibly double or more than these numbers, in Ensis siliqua, Anodonta, and Sphaerium corneum.

The presence of these cilia was first observed in transverse sections of the gill filaments of Modiolus modiolus (Text-fig. 1 A), when the appearance was assumed to be due to slight obliquity of the sections, so that part of a second large latero-frontal cell was cut. The appearance was so consistently present, however, that the correctness of this interpretation seemed highly doubtful. Careful examination of living filaments revealed the presence of a row of cilia, stouter than the frontal cilia, with certain of the characteristics of the latero-frontal cilia. These cilia have been found generally in bivalves with eu-latero-frontal cilia, including Mytilus edulis. They are more clearly discernible in some gills than in others, according largely to the transparency of the gill. For instance they were seen more clearly in the living gill of Kellia suborbicularis than perhaps in any other, except Thyasira flexuosa, though they are not particularly clear in sections of that gill (Text-fig. 1 B). In Lamellibranchs in which the outer demibranch is without a marginal groove, and the filaments are merely bent round, the various ciliary tracts are seen in profile at the bend, and the subsidiary latero-frontal cilia may occasionally be clearly seen. They are extraordinarily clear along the free edge of the outer demibranch of Thyasira flexuosa (Text-fig. 2 A, *pro-l-f.c.*). These cilia are distinguishable from the frontal cilia in successfully stained transverse sections of well-fixed material (fixed in Bouin-Duboscq's fluid: stained Heidenhain's iron haematoxylin) because they are stouter and stain more darkly, and by their darker staining ciliary rootlets. The nuclei of the cells are narrow and elongated in the direction of the length of the filament, and in cross-section appear small. They have no greater affinity for basic dyes than have the oval nuclei of the frontal cells.

So far as I am aware the only reference to these ciliated cells

is in a footnote in Engelmann's paper of 1880 (p. 511)—of which I was ignorant until after their discovery—in which he stated:
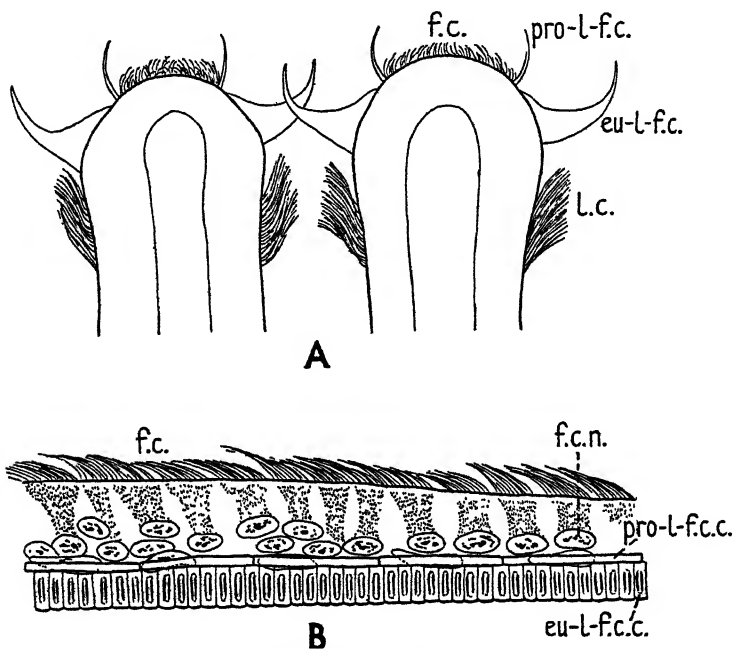
TEXT-FIG. 2 A and B.

A, Thyasira flexuosa. Two living filaments in optical section at the free edge of the outer demibranch, to show the pro-latero-frontal cilia (*pro-l-f.c.*). The frontal cilia (*f.c.*) were beating toward the observer, and therefore are not seen at their full length. *eu-l-f.c.*, eu-latero-frontal cilium. *l.c.*, lateral cilia. ×537½. B, Ensis siliqua. Sketch to show the shape of the pro-latero-frontal cells (*pro-l-f.c.c.*). *eu-l-f.c.c.*, eu-latero-frontal cell; *f.c.*, frontal cilia; *f.c.n.*, nucleus of frontal ciliated cell. The double rows of basal granules of the eu-latero-frontal cilia are shown. Bouin-Duboscq's fixative: iron haematoxylin and acid fuchsin. ×918¾. See p. 355 for Text-fig. 2 C.

'Zwischen den Eckzellen und den gewöhnlichen Flimmerzellen des Rückens ist noch eine, bisher wie es scheint übersehene, Lage sehr schmaler, in der Längsrichtung der Leistchen lang-gestreckter Flimmerzellen eingeschaltet. Sie mögen Neben-

zellen heissen. Ihr eigenthümlicher Bau stimmt wesentlich mit dem der "Seitenzellen" überein. Speciell ist die Anordung und Einpflanzung der Cilien auf der Oberfläche bei beiden die näm-
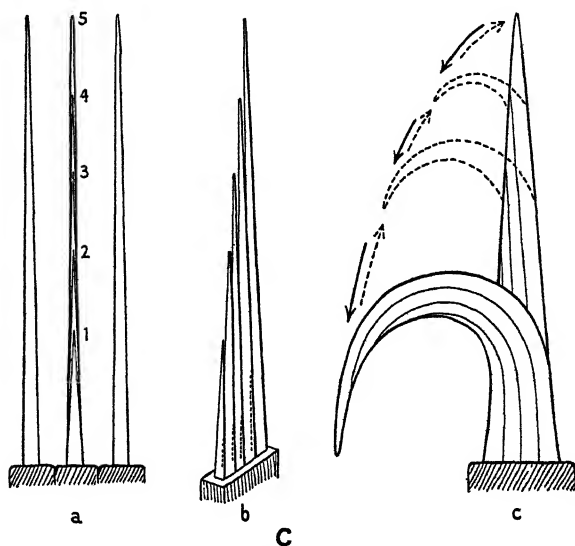
Text-fig. 2 c.

Diagrams illustrating the structure and movement of the composite eu-latero-frontal cilia, represented as being each composed of five plates only. (a) Frontal view of three cilia: plates are indicated in the middle one. (b) Oblique view of one cilium. (c) Cilium in side view to show the type of movement (after Gray, fig. 12 b, 1928, somewhat modified). The cilium is straight when at rest. The plain arrows indicate the direction of the effective stroke; the broken arrows the direction of the recovery stroke.

liche.' He figured these cells in Anodonta and Cyclas cornea.

No observations on the arrangement of the basal granules of these cells have been made for the present work, but from the living gill the cilia appear to be in a single row, and to resemble the large latero-frontal cilia in this, and the direction of the effective beat, position of rest and type of metachronal wave, rather than the lateral cilia, which they were said to do by

Engelmann. The subsidiary latero-frontal and the lateral cilia do agree, however, in being borne on narrow cells, elongated in the direction of length of the filament. The subsidiary latero-frontal cilia probably act as a second sieve, preventing the escape of small particles between the bases of the eu-latero-frontal cilia.

It is possible that Janssens (1893, p. 66) saw something of these cells in sections, for describing the latero-frontal cells of Anodonta anatina he wrote: 'Sur les coupes, on croit souvent avoir sous les yeux la section de deux rangées de cellules, Fig. 15 en bas, mais c'est une apparence produite par l'obliquité de la section ou d'autres causes.'

Thus many Lamellibranchs may be considered as possessing two rows of latero-frontal cilia on each side of the frontal surface, a row of very large and another of small or subsidiary latero-frontal cilia. The large ones may be termed e u - l a t e r o - f r o n t a l   c i l i a , and the subsidiary ones p r o - l a t e r o - f r o n t a l   c i l i a .

In N u c u l a n a and N u c u l a the nuclei in a row on the frontal side of the eu-latero-frontal cells are long and narrow—in the direction of length of the leaflets—indicating the presence of a row of long, narrow cells, such as bear the pro-latero-frontal cilia in higher Lamellibranchs, but it is extremely difficult to identify with certainty pro-latero-frontal cilia on the living leaflets of Protobranchs, owing to the thickness of the leaflets. The closeness to one another of the eu-latero-frontal cilia (about 1μ or less apart) and in N u c u l a n a the position of the latero-frontal tracts, well down on the lateral faces of the filaments (see Text-fig. 8, p. 373), add to the difficulty. A renewed and careful attempt at the definite identification of pro-latero-frontal cilia in N u c u l a   n u c l e u s was made in April 1937, and it was finally concluded that there is a regular row of cilia on the frontal side of the eu-latero-frontal cilia, corresponding to the pro-latero-frontal cilia of the higher groups, and that these cilia are most probably pro-latero-frontals. Their position of rest, however, could not be observed, and this was unfortunate, as latero-frontal cilia of whatever kind, and frontal cilia, differ in their attitude when brought to rest, latero-frontal cilia being

straight, while frontal cilia are curved. Pro-latero-frontal cilia
have not been labelled in Text-figs. 7 (p. 372) and 8 (p. 373),
which are reproductions from Part I (1936), but may be dis-
cerned in the sections of Nucula and Nuculana, though
not in those of Solenomya of which the fixation was not
particularly good. In Nucula a pro-latero-frontal cell covers
about the same length as do four or five eu-latero-frontal cells.

The Occurrence of only Small or Micro-latero-frontal Cilia.

Eu-latero-frontal cilia, such as described in the preceding
pages, are absent in certain families of Lamellibranchs, which
are characterized by the possession of small and tenuous latero-
frontal cilia. Small cilia of this kind may be termed micro-
latero-frontal cilia (*mi-l-f.c.*, Text-fig. 3 a–e). Such
families have been generally considered as lacking latero-frontal
cilia, with the exception of the Anomiidae in which they were
observed by Orton (1914), though in his paper he does not
distinguish between their size in this family and in Mytilus,
for instance. In his note-book of 1912—which he kindly allowed
me to see—he noted, however, that 'the straining cilia of
Anomia aculeata Müller (= Heteranomia squamula
(L.)) are extremely fine'.

Latero-frontal cilia have generally been considered absent in
the following bivalves: Glycymeris and Arca (Orton,
1914); Pecten (Kellogg, 1892; Janssens, 1893; Dakin, 1909;
Orton, 1914; Setna, 1930; Gutsell, 1931); Lima (Studnitz,
1931); Pinna (Atrina) rigida (Grave, 1911) and Pinc-
tada (= Margaritifera) vulgaris (Herdman, 1905).
In the work of several of these authors, however, the absence
of latero-frontal cilia is inferred, as they are not mentioned, or
not shown in the figures, but Janssens—who gave beautiful and
detailed figures of the gill structure of a number of Lamelli-
branchs—definitely stated that 'dans les diverses espèces de
Pecten que nous avons eues à l'étude, nous n'avons jamais
pu découvrir les cellules des coins', and Gutsell that 'the elongate
latero-frontal cilia described for Mytilus, Ostrea, and
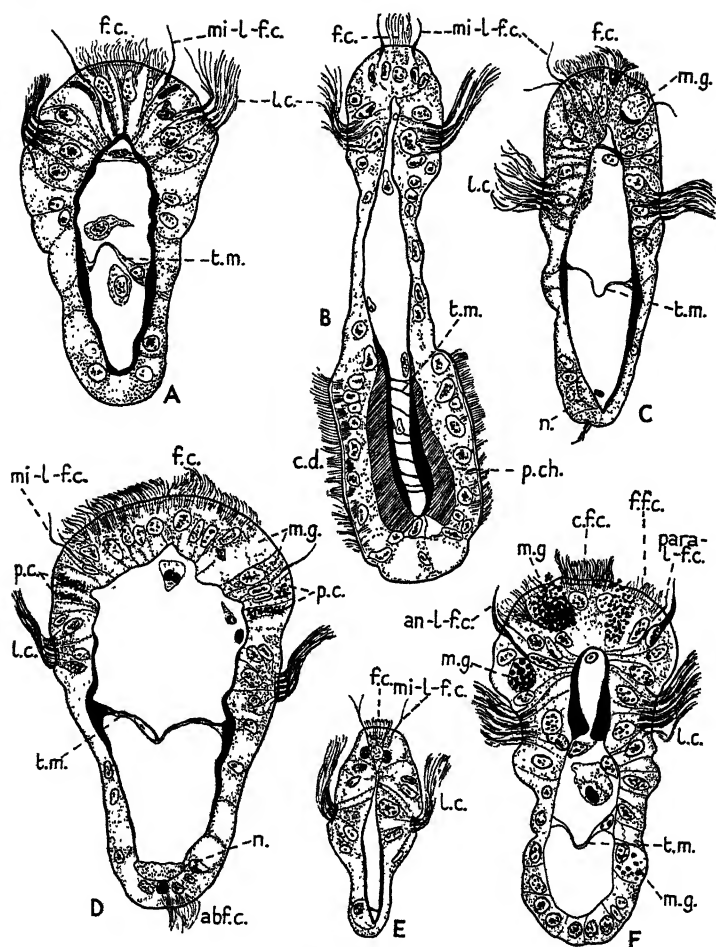
TEXT-FIG. 3.

Transverse sections of filaments of Lamellibranchs having micro-
latero-frontal cilia, and of Ostrea edulis having anomalous
together with para-latero-frontal cilia. A, Arca (Scaphula)
celox; B, Malleus albus; C, Pecten maximus (one of
the apical filaments); D, Spondylus gaederopus (one of
the two apical filaments); E, Lima hians (ordinary filament,
third from principal); F, Ostrea edulis (ordinary filament,
fifth from prinicpal). *abf.c.*, abfrontal cilia; *an-l-f.c.*, anomalous
latero-frontal cilium; *c.d.*, ciliated disc; *c.f.c.*, coarse frontal cilia;

[*For remaining description see opposite.*]

various lamellibranchs have not been found in the scallop (Pecten irradians), nor have I succeeded in demonstrating that the most lateral of the frontal cilia (in a latero-frontal position) function as would typical latero-frontal cilia'.

Curiously enough Pelseneer (1891) depicted large latero-frontal cilia in his figures of Anomia ephippium, Pectunculus (= Glycymeris) glycymeris, Arca barbata, Pecten opercularis, and Lima hians, and indeed showed them as large as in a figure of Modiolaria (=Musculus) marmoratus for instance. Hornell (1909) also figured and described large latero-frontal cilia in Placuna placenta. In Sir W. A. Herdman's slides of Placuna I find the latero-frontal cilia to be small as in Anomia (Atkins, 1936). Bourne (1907) did not show or mention latero-frontal cilia or cells in Anomia (Aenigma) aenigmatica.

In the living gill the micro-latero-frontal cilia when more or less motionless appear as a fine palisade viewed from the frontal surface. In Glycymeris glycymeris and Arca tetragona they are about 14 to 17μ long; in Heteranomia and Monia about 12μ long. Measurements of the width and breadth at the base were not made, but the cilia are very slender. In lateral view of the filament they appear as a row of shining dots, no doubt owing to bending during the stroke.

Certain details of the form of the micro-latero-frontal cilia, and the cells bearing them, have been gathered from entire filaments and from sections, which were not, however, cut for this purpose.

In the Arcidae it has been found that the cells (mi-l-f.c.c., Text-fig. 4) bearing the micro-latero-frontal cilia are narrow, elongated in the direction of length of the filament, and with long narrow nuclei (mi-l-f.c.n.). In Glycymeris glycy-

f.c., frontal cilia; f.f.c., fine frontal cilia; l.c., lateral cilia; m.g., mucous gland; mi-l-f.c., micro-latero-frontal cilium; n., nerve; p.c., pigment cells; p.ch., pale-staining chitin; para-l-f.c., para-latero-frontal cilium; t.m., transverse muscle-fibre. The chitinous skeleton is shown in black. A, B, alcohol fixation; C–F, Bouin-Duboscq's fixative; A–F, iron haematoxylin and acid fuchsin. ×735. Transverse sections of gill filaments of the Arcidae, Anomiidae, and Pteriidae have already been given (Atkins, 1936).
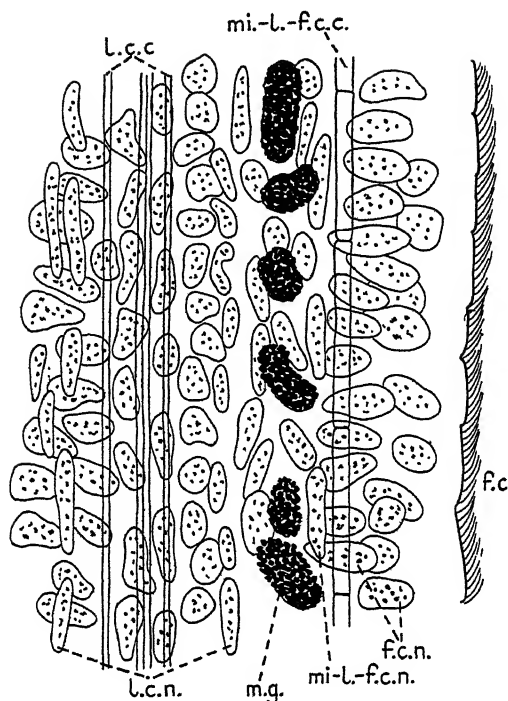
TEXT-FIG. 4.

Glycymeris glycymeris. Lateral surface of part of a filament
to show the shape of the micro-latero-frontal cells (*mi-l-f.c.c.*).
The lateral ciliated cell rows (*l.c.c.*) are also shown, though indi-
vidual cells are not indicated. As the lateral ciliated cells are
considerably larger basally than peripherally, it will be observed
that the rows of nuclei do not lie directly beneath the rows of
cells—as these appear at the surface—to which they belong. *f.c.*,
frontal cilia; *f.c.n.*, nuclei of frontal ciliated cells; *l.c.n.*, nuclei
of lateral ciliated cells; *m.g.*, mucous gland; *mi-l-f.c.n.*, nucleus of
micro-latero-frontal cell. Two per cent. osmic acid only.   × 980.

meris there are roughly about eighteen cilia to a cell: ciliary
rootlets were not seen in longitudinal sections of the filaments
in this species, nor in Arca tetragona, but were seen in
transverse sections.

In Pecten the number of micro-latero-frontal cilia to a cell
could not be determined.

In Pinna fragilis there are four small latero-frontal cilia
to a cell (Text-fig. 5 A): these will be termed provisionally micro-
latero-frontal cilia, though there is some doubt as to their
homology, owing to the possibility of there being a second row
of latero-frontal cilia present (see p. 363). Each cilium, or more
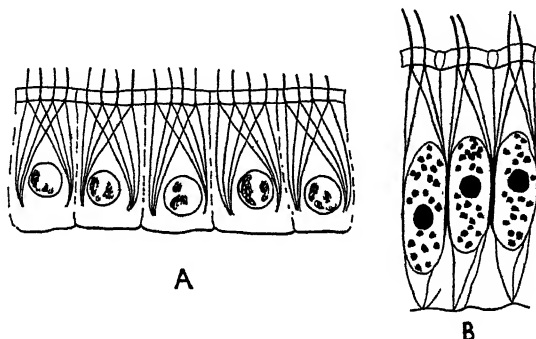
TEXT-FIG. 5.

A, Pinna fragilis. Section, nearly sagittal, through the latero-
frontal epithelium. Bouin-Duboscq's fixative; iron haematoxylin.
×1,470. B, Mytilus edulis. Sagittal section through the
latero-frontal epithelium. Fleming without acetic, iron haema-
toxylin, counterstained with orange G and silver nitrate. $\frac{1}{12}$ objec-
tive and No. 18 (Zeiss) eye-piece. B, after Bhatia, 1926.

probably each of the fibres of the cilium, has two rootlets, or
rhizoplasts, which diverge widely from a basal granule to pass
on opposite sides of the nucleus. The rootlets of the four cilia
cross one another, except the outer ones of the two outer cilia.
A similar disposition of the rootlets is found in the cells of eu-
latero-frontal cilia, for example in Mytilus, where the two
rootlets of the fibres forming the sides of a triangular plate of
the large complex cilium pass on opposite sides of the nucleus,
the two inner ones crossing each other (Text-fig. 5 B). According
to Lucas (1931) the ciliary rootlets extend to the nuclear zone
only, and not to the base of the cell, as described by Bhatia
(1926). Apparently two of the micro-latero-frontal cilia of
Pinna fragilis correspond to a single large complex latero-
frontal cilium, of Mytilus for instance. In Pinna fragilis,
however, the four cilia of each cell are entirely separate, and not

connected, as are the two fibres forming the sides of a triangular plate of the large complex cilium of the majority of Lamellibranchs. It is not impossible, though I think it unlikely, that each cilium of Pinna may consist of two rows of fibres side by side as in eu-latero-frontal cilia, but so close together that at a magnification of 1,470 diameters two sets of ciliary rootlets could not be distinguished.

The anomalous latero-frontal cilia of Ostrea (see p. 365) could be derived from micro-latero-frontal cilia as seen in Pinna by division of the cell; in such a hypothetical division two sets of basal granules with their rootlets would pass into one cell, and the other two sets of basal granules with their rootlets into the other. The two cilia of each cell might then approach each other and finally their fibres become connected by membranes. Possibly eu-latero-frontal cilia may have arisen either in this way from cilia of the micro-latero-frontal type, or as fully formed eu-latero-frontals.

The intra-cellular fibre system of epithelial cells may serve a function of co-ordination, as suggested by Worley (1934), but it is noteworthy that there is a similar complicated arrangement of the ciliary rootlets of the eu-latero-frontal cilia (e.g. in Mytilus) where the ciliary fibres linked up by these rootlets must beat synchronously—for they form the edges of a triangular element—and of the micro-latero-frontal cilia of Pinna, where the four separate cilia of a cell linked up in a similar manner probably beat metachronously. Unfortunately the beating and metachronism of the latero-frontal cilia of Pinna were not especially noticed owing to the difficulty of observing them in this form. If in Pinna the cilia on individual cells should be found to beat metachronously, then it would seem possible that the arrangement of ciliary rootlets may not be related to the type of unicellular co-ordination. Under the influence of certain drugs the metachronous beating of the cilia of individual epithelial cells may change to synchronous, but this is attributed to the harmful effect of the drugs on the regulatory mechanism, the cilia becoming uncontrolled and usually beating simultaneously due to their mechanical effect upon each other (Worley, 1934).

Although cilia with a structure closely comparable to that of

eu-latero-frontal cilia are known, for instance the velar cilia of
the Nudibranch veliger, where each cilium consists of about
fifteen plates and there are two, three, four, and sometimes more
such complex cilia to a cell (Carter, 1926), and the very long
abfrontal cilia or cirri of Mytilus, each consisting of four or
five plates in Mytilus galloprovincialis (Carter, 1924),
it is not known whether they have a similar arrangement of the
ciliary rootlets, or if the arrangement described above is peculiar
to cilia with a straining function. Carter (1928) held that a
ciliary rootlet is not a definitely differentiated portion of the
cytoplasm, and doubted the existence of such fibres in the
latero-frontal cells of Mytilus and in those of the velar cells
of Nudibranch veligers.

In thin transverse sections of the filaments of Pinna
fragilis there is occasionally an appearance as of two latero-
frontal cilia on each side of the filament. This may be due to
slight obliquity of the sections. In most bivalves an examina-
tion of the living gill is the best way in which to settle such a
question, but the living gill of Pinna is extremely difficult
to deal with owing to its deep plications, organic junctions, and
above all, extreme sensitiveness, the slightest stimulation caus-
ing it to contract to a contorted mass of filaments. If two rows
should prove to be present, then the arrangement of the ciliary
rootlets in the latero-frontal cells of Pinna as compared with
that in the Ostreidae (see p. 365) is all the more interesting. It
is possible, however, that diverging ciliary rootlets from an
entire cilium, passing on opposite sides of the nucleus as in
Pinna, or from a ciliary fibre of a triangular element as in
Mytilus, may be linked up in some way with a special type
of behaviour, and so are likely to be found in all cells the cilia of
which have this characteristic. It is therefore perhaps not
impossible that all types of straining cilia will be found to have
diverging rootlets.

In Malleus albus each latero-frontal cilium has a short
row of basal granules, rather less than 1μ long, in the plane of
the beat; this indicates that the cilia are slightly blade-shaped,
that is, they are wider in the plane of the beat than in the plane
perpendicular to it. The material from which the sections were

cut had been preserved in alcohol, and probably because of the resulting poor fixation the ciliary rootlets could not be distinguished in longitudinal sections through the latero-frontal epithelium. There appeared to be about four cilia to a cell, but this could not be definitely determined. The width of these cilia at the base—unusual for micro-latero-frontals—suggests that there is just a possibility, as in Pinna fragilis, of two rows of latero-frontal cilia, though a second row was not discernible in sections, and in the state of preservation this was hardly to be expected.

The latero-frontal cilia themselves are less close together in Pinna fragilis and Malleus albus than in Glycymeris and Arca, and are fewer to a cell, and somewhat larger. Owing to the possibility of there being two rows of latero-frontal cilia in Pinna fragilis and Malleus albus details of the structure of the latero-frontal cilia in these two species have been omitted from Table I, p. 370.

Lucas (1931) noted for Mytilus edulis and Amblema costata that in fixed and stained preparations the nucleus of the eu-latero-frontal cells has a stronger affinity for basic dyes than have the nuclei of other cells of the gill. This was found to be so in most of the gills sectioned for the present work, but not invariably. In most bivalves with micro-latero-frontal cells there seems to be little, if any, difference in affinity for basic dyes between the nuclei of these cells and others, but the Indian fresh- and brackish-water form, Arca (Scaphula) celox, is an apparent exception in which the nuclei in cross-section are narrow and stain darkly (Text-fig. 3 A). The micro-laterofrontal cilia in this species of Arca (museum material preserved in alcohol) showed more clearly than in well-fixed material of other forms.

Lamellibranchs with micro-latero-frontal cilia, with the possible exception of Pinna, appear to be without a subsidiary row, such as is present in bivalves with eu- and in those with anomalous latero-frontal cilia.

Micro-latero-frontal cilia have been found to possess certain of the characteristics of eu-latero-frontal cilia, namely:

(1) their arrangement in a single, regular row;

(2) the direction of the effective beat, which is toward the frontal surface;

(3) their position of rest;

(4) the same type of metachronal wave. The metachronal wave was especially noted in the Arcidae, Anomiidae, and Pectinidae.

They differ from them, however, in their (a) smaller size, especially in the width and breadth at the base; (b) apparently simpler structure, being probably composed of fibres and not of triangular plates; and (c) in that more than one is borne on a cell, while there is only a single eu-latero-frontal cilium to a cell.

Micro-latero-frontal cilia agree closely with the pro-latero-frontal cilia of bivalves possessing eu-latero-frontal cilia (see Table I, p. 370): they may be actively straining cilia, or may perhaps act merely as a guard to prevent loss of food particles from the frontal tract.

THE OCCURRENCE OF MODERATE-SIZED OR ANOMALOUS LATERO-FRONTAL CILIA, TOGETHER WITH SUBSIDIARY OR PARA-LATERO-FRONTAL CILIA IN ONE FAMILY ONLY, THE OSTRE-IDAE.

In one family, the Ostreidae, the latero-frontal cilia of the main row are only moderately developed, especially as to their size at the base, and may be termed anomalous latero-frontal cilia (an-l-f.c., Text-fig. 3 F, p. 358). They are however about 14 to 25μ long.

The cells from which these cilia arise are small and bear one cilium each, as may be seen when the cells are becoming dissociated under the influence of the narcotic stovaine. These cilia appear to have the same structure, and the same arrangement of their ciliary rootlets as in Mytilus, but, while being blade-shaped, they are much less wide at the base in the plane of the beat (roughly about $1\frac{1}{4}\mu$), than those of Mytilus galloprovincialis (4–5μ, see Carter, 1924) and other bivalves having eu-latero-frontal cilia (see eu-l-f.c., Text-fig. 1, p. 351, and an-l-f.c., Text-fig. 3 F). The effective beat is in the same direction and the position of rest is the same as that of the

other forms of latero-frontal cilia, and the metachronal wave is of the same type.

The eu-latero-frontal cilia of bivalves are characteristically, closely, and evenly spaced; those of E n s i s   s i l i q u a for example are about 2μ apart, those of N u c u l a   n u c l e u s about 1μ apart. In O s t r e a   e d u l i s, O s t r e a   v i r g i n i c a, and O s t r e a   a n g u l a t a it has been found that the length of the anomalous latero-frontal cilia and their distance apart varies on different filaments. They are longest and closest together on the principal and transitional filaments, and shortest and farthest apart on the filaments forming the plical crests. Frequently they are closer together on that side of the transitional filament next to the principal than on the side away from it. In O s t r e a   e d u l i s and O s t r e a   v i r g i n i c a the distance apart of these cilia varies between about 1·5 to 3·7μ, while on the filaments of the plical crests of O s t r e a   a n g u l a t a they are about 6 or 7μ apart.[1] In the genus O s t r e a the frontal currents on the principal and transitional filaments are dorsal in direction, and particles intended for consumption are mainly carried along these. That the latero-frontal cilia, which are straining cilia preventing particles from being swept through to the exhalent chamber, should be longer and closer together on these filaments than on those forming the plical crests, which are concerned chiefly with rejection (Atkins, 1937), is indicative of functional correlation.

While the anomalous latero-frontal cilia of O s t r e a are easily seen in fresh material, they are difficult to distinguish in sections

[1] An interesting instance of wider spacing of eu-latero-frontal cilia on one side of a filament than on the other was observed over stretches of the filaments of P e t r i c o l a   p h o l a d i f o r m i s, due to the presence of a protozoon 35 to 80μ long, adhering by the whole length of its body parallel to the filament in the region between the lateral and latero-frontal cilia, and suppressing some of the latero-frontal cilia, so that they were about twice as widely spaced as normally; while in some places they were entirely wanting for short stretches. The parasites, which were very numerous, were frequently attached along one side only of a filament, the opposite side where the latero-frontal cilia were normally spaced being free from them. This parasite though allied to the Ciliata appeared, in the stage seen, to be without cilia.

(see also Yonge, 1926): this is no doubt owing to the fact that under the action of fixatives they tend to separate into their constituent fibres, and, as the cilia are only of moderate size, the tufts of fibres are not very noticeable. They were found to retain their form better in sections of material fixed in 2 per cent. osmic acid than in Bouin-Duboscq's fluid.

Subsidiary latero-frontal cilia are present in the Ostreidae, but are very difficult to distinguish even in the living gill. They are possibly somewhat smaller than those of bivalves possessing eu-latero-frontal cilia; however, the gills are rather opaque and the cilia therefore difficult to observe even in a preparation of a single lamella. Apart from the organic junctions, the presence of numerous blood cells in the lacunae of the demibranch tends to make it opaque, especially when these are greenish.

As will be seen from the second half of this paper (p. 383), it is probable that the anomalous latero-frontal cilia of the Ostreidae are not homologous with eu-latero-frontal cilia, and as the homology of the subsidiary latero-frontal cilia of this family with the pro-latero-frontal cilia of forms possessing eu-latero-frontals is doubtful, it is proposed to call them para-latero-frontal cilia. In Text-fig. 1 of Part II (1937), therefore, these cilia should have been labelled *para-l-f.c.* and not *pro-l-f.c.* Para-latero-frontal cilia have been omitted from Table I, p. 370, for, from the little that is known of them, they appear to agree in structure and arrangement with pro- and micro-latero-frontal cilia.

To summarize: anomalous latero-frontal cilia agree with eu-latero-frontal cilia in being borne singly on small cells, of which the greater diameter is at right angles to the length of the filament; in having, so far as is known, a similar structure and a similar arrangement of their ciliary rootlets; and in the direction of the effective beat, position of rest, and type of metachronal wave. They also agree in being accompanied by subsidiary latero-frontal cilia.

They differ from eu-latero-frontal cilia, however, in their smaller size, especially as to the width at the base in the plane of the beat, inconspicuousness in sections, and marked variation in length and spacing on different filaments of the same individual.

Lamellibranchs with Eu-latero-frontal Cilia.

In 1891 Pelseneer depicted large latero-frontal cilia in all thirteen of the lamellibranch gills he figured in his 'Contribution à l'étude des Lamellibranches', including those of five species, Anomia ephippium, Pectunculus (= Glycymeris) glycymeris, Arca barbata, Pecten opercularis, and Lima hians in which large ones are undoubtedly absent. Kellogg (1892) soon after stated that they were absent in Solenomya velum and Pecten irradians—in the first instance almost certainly incorrectly—and expressed the opinion that latero-frontal cilia are 'not so widely found among Lamellibranchs, I believe, as seems to be so generally supposed'. Ridewood (1903), in the course of his extensive work on the gills of Lamellibranchs, found them to occur in the great majority (his remarks on these cilia are based presumably on preserved material only), and this has been borne out in the present work, mostly on living gills. As late as 1930, however, Nicol referred to species possessing latero-frontal cilia—that is eu-latero-frontals —as 'aberrant Lamellibranchs'.

During the course of the present work, large or eu-laterofrontal cilia were found in members of the three families of Protobranchia (they were previously recorded by Orton, 1912, 1914, in Nucula and Solenomya togata); in all the marine families of Eulamellibranchia obtainable at Plymouth, and in the fresh-water families Dreissensiidae, Sphaeriidae, Unionidae, Mutelidae, and Aetheriidae. In the Filibranchia they were found in the Mytilidae and Trigoniidae only. They were absent in all the Pseudolamellibranchia examined. A list of the species of Lamellibranchia found to have eu-laterofrontal cilia is given on p. 374. Pro-latero-frontal cilia are perhaps always present also, though they have not always been recognized owing to difficulties of observation or imperfect preservation of material.

A visual impression is that there is little or no direct correlation between the size of the bivalve, gill, or filament, and that of the eu-latero-frontal cilia. There is no great difference in the size of these cilia in Modiolus modiolus, which may reach

a length of 11 cm. or more, and in Kellia suborbicularis, of which the largest specimen seen was about 1 cm. long. The depth of a filament, from the frontal to the abfrontal surface, of Modiolus modiolus is more than four times that of one of Kellia suborbicularis, while the frontal surface is about three times wider, antero-posteriorly, as shown in Text-fig. 1 A and B (p. 351).

While a certain amount of variation in the size of eu-latero-frontal cilia is found when actual measurements are taken, there is no possibility of confounding them with micro-latero-frontal cilia. (A provisional comparison of the different types of latero-frontal cilia is given in Table I.)

Among fresh-water forms, Lampsilis and Quadrula are stated by Grave and Schmitt (1925) to have latero-frontal cilia which often exceed 15μ in length, and are about 1 to $1\frac{1}{2}\mu$ in diameter at the base. While this is unusually small for eu-latero-frontal cilia, the size at the base is sufficient to prevent their being confounded with micro-latero-frontal cilia. Measurements were made of these cilia in a few fresh-water forms during the present investigation. Dreissensia polymorpha was found to have eu-latero-frontal cilia about 24μ long, and roughly about 9μ wide at the base in the plane of the beat, measured living. Those of Aetheria elliptica are about 27μ long (measured from preserved entire filaments), though they appear somewhat less than this in transverse section (Text-fig. 6 A), and are about 6 or 7μ wide at the base in the plane of the beat (measured from sections); those of Mutela bourguignati (Text-fig. 6 B), judging from sections, are about 20μ long, and about 6μ wide. Measurements made on sections, however, are unreliable, as apart from the occurrence of shrinkage, the entire length of the cilium may not be cut.

Among marine forms Carter (1924) gave their length in living Mytilus galloprovincialis as about 30μ, and at the base $\frac{3}{4}$ to 1μ broad in the plane perpendicular to the beat, and 4 to 5μ wide in the plane of the beat. The only measurements I have made of eu-latero-frontal cilia of marine forms were in Gastrochaena dubia, in which these cilia are roughly about 26μ long and 6 to 8μ wide at the base in the plane of the beat

TABLE I.   *Provisional Comparison of Types of Latero-frontal Cilia.*

	Eu-latero-frontal Cilia.	Anomalous latero-frontal Cilia.	Micro-latero-frontal Cilia.*	Pro-latero-frontal Cilia.*
Cells.	Small: longer diameter at right angles to length of filament.	As eu-latero-frontal cilia.	Long, narrow: elongated in the same direction as flament.	As micro-latero-frontal cilia.
Number of cilia to a cell.	1		More than 1: about 18 in Glycymeris glycymeris.	A number to each cell (see p. 352).
Structure of cilium.	Composed of a number of triangular plates (10–15 in Mytilus according to Carter, 1924), bounded by fibres on each side of the cilium. Surface of plates at right angles to plane of beat: plates shorter on side of cilium which is directed forward in effective beat. At the base the cilium is considerably wider in the plane of the beat than at right angles to it, so is characteristically broadly triangular in side view.	Probably as eu-latero-frontal cilia, though they are not as large.	Probably no plates, but each cilium composed of a few fibres, probably placed one behind the other in the plane of the beat, so that the cilium is slightly wider in this plane, than in that at right angles to it.	Probably no plates, but each cilium composed of a few fibres, probably placed one behind the other in the plane of the beat. The difference in the width and breadth at the base is slight.
Basal granules.	Each plate associated with a pair, so that in surface view of the cell there is a double row of basal granules at right angles to the length of the filament.	Probably as eu-latero-frontal cilia.	The arrangement was not observed.	The arrangement was not observed.
Ciliary rootlets.	Two diverge from each basal granule, and pass on opposite sides of the nucleus, there being two pairs to each plate.	Probably as eu-latero-frontal cilia.	The arrangement was not observed.	The arrangement was not observed.
Length of cilium.	About 20–80 μ (Lampsilis and Quadrula often exceed 15 μ).	14–25 μ.	About 12 μ in Heteranomia and Monia: 14–17 μ in Glycymeris and Arca.	About 10 μ in Modiolus modiolus, and 6 μ in Kellia suborbicularis (measured from sections).
Width of cilium at base in the plane of the beat.	About 4–9 μ, excluding those of Lampsilis and Quadrula which are said to be 1–1¼ μ in diameter, and of Xylophaga which are about 2 μ wide.	Roughly about 1¼ μ, measured from sections.	Not measured, but probably not much greater than at right angles to the plane of the beat.	Not measured, but probably not much greater than at right angles to the plane of the beat.

* Para-latero-frontal cilia appear to agree with micro- and pro-latero-frontal cilia in the little that is known of them.
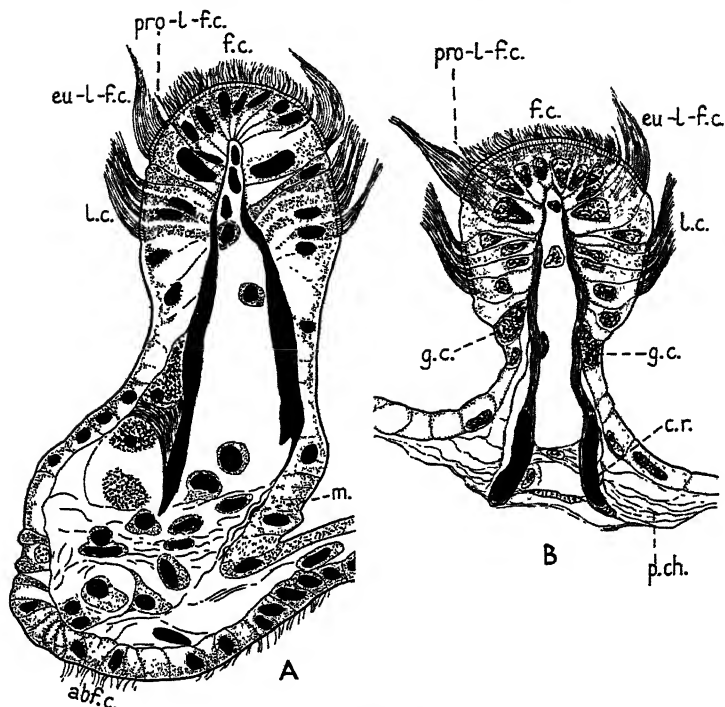
TEXT-FIG. 6.

Transverse sections of the filaments of two fresh-water Lamelli-
branchs, having eu-latero-frontal cilia. A, A e t h e r i a e l l i p t i c a;
B, M u t e l a b o u r g u i g n a t i (inner demibranch). *abf.c.*, ab-
frontal cilia; *c.r.*, calcified rod; *eu-l-f.c.*, eu-latero-frontal cilium;
f.c., frontal cilia; *g.c.*, gland-cell; *l.c.*, lateral cilia; *m.*, muscle-
fibres; *p.ch.*, pale-staining fibrous chitin; *pro-l-f.c.*, pro-latero-
frontal cilium (?). The chitinous skeleton is shown in black in
A e t h e r i a, but in M u t e l a it is shaded and the calcified rods
shown in black. Alcohol fixation; iron haematoxylin and acid
fuchsin. Owing to the condition of fixation pro-latero-frontal
cilia could not be identified with certainty. ×735.

(measured living), and in X y l o p h a g a d o r s a l i s, in which
they are roughly 20μ long and only 2μ wide at the base in the
plane of the beat (measured from sections). This narrowness
of the eu-latero-frontal cilia in X y l o p h a g a is not found in
the allied B a r n e a p a r v a, and seems exceptional among
marine forms.
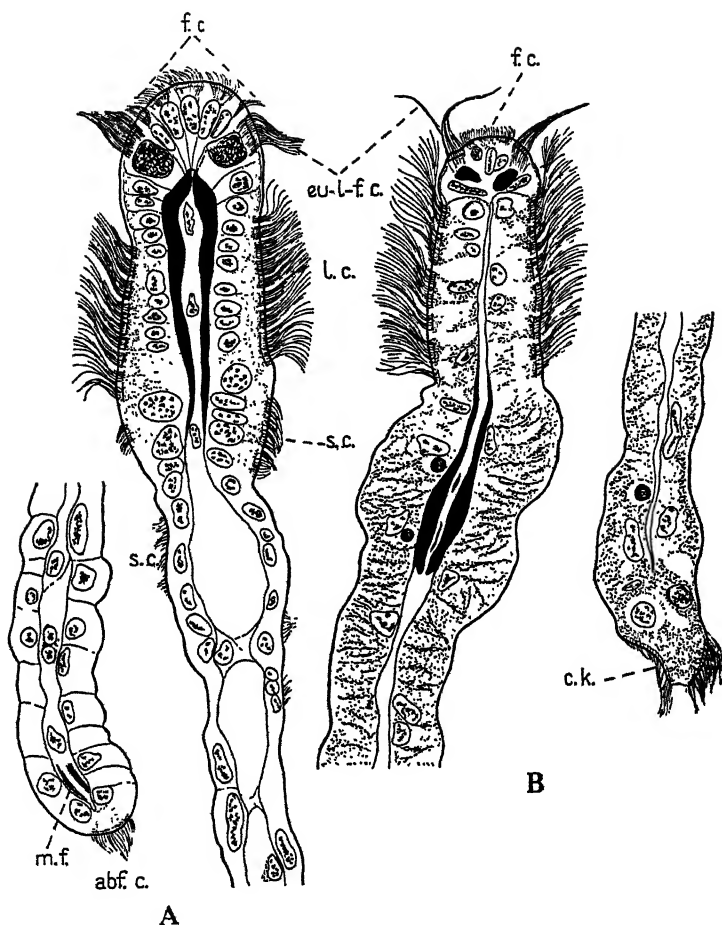
TEXT-FIG. 7.

Transverse sections of the gill leaflets of two Protobranchs. A,
Nucula; B, Solenomya togata. *abf.c.*, abfrontal cilia;
c.k., ciliated knob, or glandular organ; *eu-l-f.c.*, eu-latero-
frontal cilia; *f.c.*, frontal cilia; *l.c.*, lateral cilia; *m.f.*, muscle-fibres;
s.c., slight ciliation dorsal to the lateral cilia. Bouin-Duboscq's
fixative; iron haematoxylin. ×735.

In the Protobranchia there is a certain amount of variation
in the shape of the eu-latero-frontal cilia. In Nucula they are
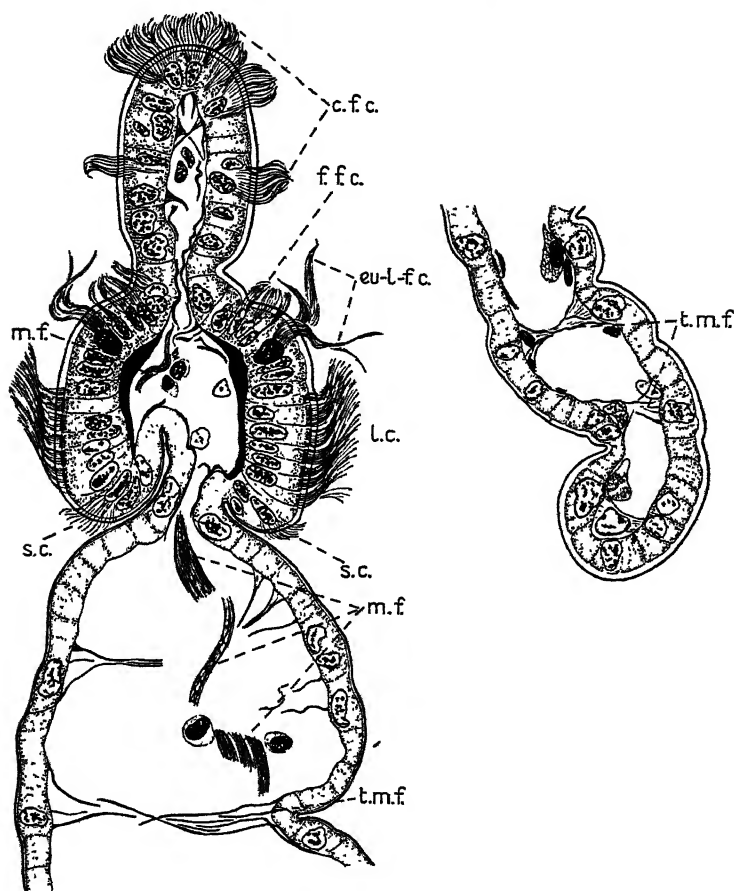
TEXT-FIG. 8.

Transverse section of a gill leaflet of the Protobranch Nuculana minuta. The whole is not figured because of the great depth (frontal to abfrontal) of the leaflets. The involution of the walls near the lateral ciliated tracts is due to contraction of the muscles. *c.f.c.*, coarse, and *f.f.c.*, fine frontal cilia; *eu-l-f.c.*, eu-laterofrontal cilia; *l.c.*, lateral cilia; *m.f.*, radiating striated muscle-fibres; *s.c.*, slight ciliation dorsal to the lateral cilia; *t.m.f.*, transverse muscle-fibres. Bouin-Duboscq's fixative; iron haematoxylin. ×735.

rather short, but very broadly triangular in side view (Text-fig. 7 A); while in Nuculana minuta (Text-fig. 8) and

Solenomya togata (Text-fig. 7 B) they are longer, but less broadly triangular. In Nuculana minuta they are about 30μ long, and in Nucula nucleus about 20μ long, measured living.

What is especially characteristic of the size of eu-latero-frontal cilia, is not so much the length, which apparently may vary from 15 to 30μ at least, but their breadth at the base in a plane perpendicular to the beat, and more especially their relatively great width in the plane of the beat; this last dimension giving them their distinctively triangular shape in side view.

List of Lamellibranchs found[1] to have Eu-latero-frontal Cilia.

PROTOBRANCHIA.

Solenomyidae: Solenomya togata.[2]

Nuculidae: Nucula nucleus (L.), Nucula radiata Hanley, Nucula nitida Sowerby.

Nuculanidae: Nuculana (= Leda) minuta (Müller), Nuculana pella.[2]

FILIBRANCHIA.

Trigoniidae: Trigonia margaritacea.[2]

Mytilidae: Mytilus edulis L., Modiolus modiolus (L.), Modiolus adriaticus Lamarck, Modiolus phaseolinus (Philippi), Musculus (= Modiolaria) marmoratus (Forbes), Musculus (= Crenella) discors (L.).

EULAMELLIBRANCHIA.

Dreissensiidae: Dreissensia polymorpha (Pallas).

Astartidae: Astarte sulcata (da Costa).

Thyasiridae: Thyasira flexuosa (Montagu).

Lucinidae: Myrtea spinifera (Montagu), Phacoides borealis (L.).

Ungulinidae: Diplodonta rotundata (Montagu).

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[1] The list is comprehensive, but does not include the examination of members of all families of Lamellibranchs.

[2] Preserved material only examined.

Erycinidae: Kellia suborbicularis (Montagu), Lasaea rubra (Montagu).

Galeommatidae: Galeomma turtoni Sowerby.

Leptonidae: Lepton squamosum (Montagu).

Montacutidae: Montacuta ferruginosa (Montagu), Mysella bidentata (Montagu), Entovalva perrieri (Malard).

Cyprinidae: Cyprina islandica (L.).[1]

Sphaeriidae: Sphaerium corneum (L.).

Unionidae: Anodonta anatina (L.).

Aetheriidae: Aetheria elliptica,[1] Mülleria daleyi.[1]

Mutelidae; Mutela bourguignati.[1]

Cardiidae: Cardium echinatum L., Cardium ovale Sowerby, Cardium edule L., Cardium crassum Gmelin (=norvegicum).

Veneridae: Dosinia exoleta (L.), Dosinia lupinus (L.), Gafrarium minimum (Montagu), Venus verrucosa L., Venus casina L., Venus ovata Pennant, Venus fasciata (da Costa), Venus striatula (da Costa), Paphia (=Tapes), rhomboides (Pennant), Paphia pullastra (Montagu), Paphia decussata (L.).

Petricolidae: Petricola pholadiformis Lamarck, Mysia undata (Pennant).

Donacidae: Donax vittatus (da Costa).

Tellinidae: Tellina tenuis da Costa, Tellina fabula Gmelin, Tellina donacina L., Tellina crassa Pennant, Macoma balthica (L.).

Semelidae: Scrobicularia plana (da Costa) (=piperata), Abra alba (S. Wood), Abra nitida (Müller).

Asaphidae: Gari fervensis (Gmelin) (=ferroensis), Gari tellinella (Lamarck).

Solenidae: Solen marginatus Montagu (=vagina), Ensis siliqua (L.), Ensis arcuatus (Jeffreys), Ensis ensis (L.), Cultellus pellucidus (Pennant).

Solecurtidae: Solecurtus scopula (Turton) (=candi-

[1] Preserved material only examined.

dus), Solecurtus chamasolen (da Costa) (=anti-quatus).

Mactridae: Mactra corallina (L.), Spisula elliptica (Brown), Spisula solida (L.), Spisula subtruncata (da Costa).

Lutrariidae: Lutraria lutraria (L.) (=elliptica).

Myidae: Mya truncata L.

Erodonidae: Aloidis gibba (Olivi) (=Corbula nucleus).

Hiatellidae: Hiatella arctica (L.), Hiatella gallicana (Lamarck) (=rugosa).

Gastrochaenidae: Gastrochaena dubia (Pennant).

Pholadidae: Barnea candida (L.), Barnea parva (Pennant), Pholadidea loscombiana Turton, Xylophaga dorsalis Turton.

Teredinidae: Teredo navalis L.

Periplomatidae: Cochlodesma praetenue (Montagu).

Thraciidae: Thracia villosiuscula (Macgillivray), Thracia distorta (Montagu).

Lyonsiidae: Lyonsia norwegica (Gmelin).

Pandoridae: Pandora pinna (Montagu) (=obtusa).

Lamellibranchs with Micro-latero-frontal Cilia.

In Lamellibranchs in which micro-latero-frontal cilia occur, the frontal cilia frequently extend round on to the lateral faces of the filaments, and, therefore, the micro-latero-frontal cilia are actually lateral in position, thus increasing the difficulty of discerning these tenuous cilia when a filament is viewed from the side. A certain amount of shrinkage, however, occurs on fixation, so that in transverse section they are more or less latero-frontal in position (Text-fig. 3, p. 358).

In successfully stained transverse sections of well-fixed material the micro-latero-frontal cilia themselves can frequently be seen, and nearly always they can be distinguished from the frontal cilia by their more conspicuous basal granules and darker staining ciliary rootlets. But by far the best way to determine their presence is in the living gill. When living material was unobtainable, and where fixation was far from perfect, as in

alcohol-preserved museum specimens, it seemed justifiable to deduce the presence of micro-latero-frontal cilia when eu-latero-frontals were clearly absent. Entire filaments were examined unstained in alcohol, or formalin, with a drop or two of glycerine added, and the results obtained verified by sectioning. In entire filaments the presence of eu-latero-frontal cilia is generally easily determinable, unless the material is in very poor condition. The arrangement and size of the nuclei of the latero-frontal cells was also taken into consideration. When eu-latero-frontal cilia are present the row of large, closely set nuclei is conspicuous, and the absence of such a row was taken as confirmative evidence of the absence of eu-latero-frontal cilia. The nuclei of the micro-latero-frontal cells, which are elongated in the direction of length of the filament, and are widely spaced, are inconspicuous in the entire filament. It is not impossible that the presence of moderate-sized or anomalous latero-frontal cilia, as in Ostrea, might be overlooked, though the presence of a row of small, closely set nuclei, as in that genus, should be evident at least on wide principal filaments.

Micro-latero-frontal cilia are difficult to distinguish even in living gills until one has become accustomed to them, and it is understandable that their presence escaped workers restricted to preserved material. In 1931 on a first examination of Heteranomia squamula (L.) (=Anomia aculeata Müller) latero-frontal cilia were thought to be absent, and it was only in view of Orton's (1914, p. 299) published statement of their presence (his notebook in which they are described as 'extremely fine' had not then been seen, and was not until September 1932), that a careful search was made and they were discovered in a reduced form. The difference in size of the large latero-frontal cilia of many Lamellibranchs and the tenuous ones of Heteranomia was so striking, that it seemed possible that small ones had been overlooked in Pecten, and other species, by workers familiar with them in their fully developed form, and it was decided to examine the bivalves available, with the results given in this paper.

Micro-latero-frontal cilia would seem to be straining cilia, or at least to act as a guard to prevent loss of food particles from

the frontal tract, but their effectiveness as such is presumably less than that of the large ones, and very much less in Hetera-nomia and Monia (Atkins, 1936).

Micro-latero-frontal cilia have been found in the following families: Arcidae, Anomiidae, Pteriidae, Pectinidae, Spondy-lidae, Limidae, Pinnidae, and are inferred to be present in the Amussiidae (Atkins, 1938). They are also inferred to be present in the Isognomonidae and Vulsellidae (see p. 376). A list of species is given on p. 379.

The distribution in the Lamellibranchia of species having micro-latero-frontal cilia is of great interest. The form of these cilia does not appear to be correlated with habitat, for bivalves possessing them are adapted to a variety of environments. Some are shallow water forms; others have a wide vertical range. Some live in comparatively clear water; others in situations where sediment is liable to be heavy. A number are attached by a byssus, temporarily (Arca, Pinctada, Malleus, Isognomon, Pinna, and certain Pectinidae and Limidae to rocks and stones; Pteria to Eunicella), or permanently (Anomia, Heteranomia, and Monia); others are ce-mented to rock (Chlamys distorta, Spondylus, and Plicatula). Among these attached forms Pinna is perhaps alone in being able to burrow by means of a strong water-current (Grave, 1911). Placuna and Glycymeris are unattached. According to Hornell (1909) 'the bottom favoured by Placuna placenta is a fairly stiff or pasty greyish-black mud. On this the shells generally lie prone upon their convex left valves, the hinge region sometimes slightly sunk in the mud, which may lightly cover the dorsal third of the shell.' Glycymeris glycymeris burrows by means of its foot in sand and shell gravel, lying more or less hidden beneath the surface (Atkins, 1936). The two burrowing members of the group do not employ the same methods: the free living, circular valved Glycymeris burrows chiefly by means of its foot, though with the aid of a current expelled from the anterior region of the shell; the byssi-ferous, triangular shaped Pinna by means of a water-current expelled from the pointed anterior end of the shell (see Grave, 1911); this sharply pointed extremity is no doubt of assistance

in penetrating the mud and soft sand in which the animal lives. Many of the Pectinidae and Limidae, and the Amussiidae are free swimming; Lima hians is nidamentous. Vulsella lives embedded in sponges.

There thus appears to be no correlation between habitat or mode of life and the possession of micro-latero-frontal cilia, but, as will be seen later (p. 383), it is considered that these cilia indicate the genetic relationship of families possessing them.

List of Lamellibranchs found to have Micro-latero-frontal Cilia.

FILIBRANCHIA.

Arcidae: Arca tetragona Poli, Arca lactea L., Arca (Scaphula) celox Benson,[1] Glycymeris glycymeris (L.).

Anomiidae; Anomia ephippium L.,[1] Heteranomia squamula (L.), Monia patelliformis (L.), Monia squama (Gmelin), Placuna placenta.[1]

PSEUDOLAMELLIBRANCHIA.

Pteriidae: Pteria hirundo (L.) (=Avicula tarentina), Pinctada (=Margaritifera=Meleagrina) margaritifera (L.),[1] Pinctada vulgaris (Schumacher),[1] Malleus albus Lamarck[1] (see, however, p. 363).

Vulsellidae: Vulsella sp.[1] (most probably).

Isognomonidae: Isognomon alata[1] (most probably).

Pinnidae: Pinna fragilis Pennant (see, however, p. 361).

Pectinidae: Pecten maximus (L.), Chlamys distorta (da Costa) (=pusio), Chlamys vitrea (Gmelin),[1] Chlamys opercularis (L.), Chlamys tigerina (Müller).

Amussiidae: Amussium pleuronectes L.[1] (most probably).

Spondylidae: Spondylus gaederopus,[1] Spondylus sp.[1] (from Great Barrier Reef).

[1] Preserved material only examined.

Limidae: Lima hians (Gmelin), Lima loscombi Sowerby.

LAMELLIBRANCHS WITH ANOMALOUS LATERO-FRONTAL CILIA.

Anomalous latero-frontal cilia, as previously mentioned, have been found in only one family of Lamellibranchs, the Ostreidae, which were placed by Pelseneer (1911) in the Pseudolamellibranchia. The species examined were Ostrea edulis L., Ostrea virginica Gmelin, and Ostrea angulata (Lamarck).

THE ARRANGEMENT OF THE VARIOUS CILIARY TRACTS ON THE GILL FILAMENTS.

(a) In Lamellibranchs with Eu-latero-frontal Cilia.

The common arrangement of the various ciliary tracts of the gill filaments in bivalves with eu-latero-frontal cilia is that shown in transverse sections of Modiolus modiolus, Kellia suborbicularis (Text-fig. 1 A–B, p. 351), Mutela bourguignati and Aetheria elliptica (Text-fig. 6, p. 371). Abfrontal cilia may or may not be present (*abf.c.*, Text-fig. 6).

In two Protobranch families, Nuculidae and Solenomyidae, the arrangement is closely comparable, except that the tracts of lateral cilia are very wide, 26μ[1] in Nucula radiata and 30μ in Solenomya togata (Text-fig. 7 A–B, p. 372). A wide tract of lateral cilia is possibly primitive, for it is found in certain Gastropods, for instance in the Rhipidoglossae, Fissurella graeca L., Trochus cinerarius L. (Pelseneer, 1891), Pleurotomaria beyrichii (Woodward, 1901), and Haliotis tuberculata (Crofts, 1929), and in the Pectinibranch, Crepidula fornicata (Orton, 1912). In Nucula the unciliated tract between the lateral and eu-laterofrontal cilia is of some width; in Solenomya togata it is narrow, and the tips of the lateral cilia extend to the level of the frontal surface (Text-fig. 7 B). Kellogg (1892, Pl. XCI,

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[1] Measured from sections parallel with the surface of the cells; in living filaments they are probably wider.

fig. 77) showed the lateral and frontal cilia as continuous in Solenomya, but this was corrected by Orton (1913).

In the Protobranch family Nuculanidae the lateral ciliated tracts, six or seven cells wide (measuring 18 to 22μ in sections of Nuculana minuta), are far from the frontal surface (*l.c.*, Text-fig. 8, p. 373), but owing to the great depth (frontal to abfrontal) of the leaflets, they are yet much closer to it than to the abfrontal surface. Close to the eu-latero-frontal cilia (*eu-l-f.c.*) is a tract of fine frontal cilia (*f.f.c.*): between these and the coarse frontal cilia (*c.f.c.*), the main tract of which is actually more or less on the frontal surface, is an unciliated region. A description of the ciliation of the gill leaflets of Nuculana minuta has already been given (Atkins, 1936).

The arrangement of the ciliated tracts in Trigonia margaritacea (Text-fig. 1 c, p. 351) is unlike that found in any other Filibranch or Eulamellibranch, resembling rather that in the Protobranch Nuculana minuta (Text-fig. 8), though with certain differences. It is not suggested, however, that there is any close relationship between Trigonia and Nuculana, which is a considerably specialized Protobranch (Atkins, 1936). The four rows of lateral cilia (*l.c.*) are far distant from the frontal surface, being in fact slightly nearer the abfrontal than the frontal surface (as in the Gastropod Crepidula (Orton, 1914)). Separated from the lateral cilia by one or two unciliated cells are the large latero-frontal cells, bearing large and long cilia (*eu-l-f.c.*). Both cells and cilia have the characteristic appearance found in numerous Lamellibranchs. Determination of the presence or absence of pro-latero-frontal cilia was impossible owing to poor fixation. The cilia clothing the frontal surface are remarkably long (*c.f.c.*); they are shown in the figure at about their full length, though they are generally cut short in sections. In a latero-frontal position are long cilia (*f.c.l.*), which, judging by their attitude in sections, beat mainly across the length of the filament, though it was impossible to determine the direction of the effective beat. From an examination of the entire filament it was seen that they are borne on three or four rows of long narrow cells, the cells being elongated in the direction of length of the filament. These cilia may

possibly send particles toward the frontal surface (that is if the effective beat should prove to be toward that surface), as do the scattered patches of coarse cilia in Nuculana minuta (Atkins, 1936); or (if the effective beat should prove to be abfrontal in direction) they are perhaps additional current producing, that is functionally lateral, cilia: an examination of living material is the best way to determine this. Between these cilia and the true latero-frontal cilia, the surface of the filament appears to be uniformly ciliated (*f.f.c.*), though it is impossible to be certain of this owing to the imperfect fixation. The cilia are finer, shorter, and not as closely set as those of the frontal surface. By analogy with certain other bivalves, it seems possible that the long, stout cilia of the frontal surface will be found to transport unwanted particles ventrally, while the fine ones, clothing the sides of the filaments between the coarse frontal and the latero-frontal cilia, will be found to convey particles which are destined to be eaten. The frontal currents on the gills of this species are likely to be of much interest.

Ridewood (1903, p. 202) stated that the frontal and lateral cilia of Trigonia lamarki are normal. Unless the arrangement of the ciliated tracts of the gill filaments differs widely in the two species, Trigonia margaritacea and Trigonia lamarki, he evidently included the true laterofrontal cilia (*eu-l-f.c.*, Text-fig. 1 c) with the lateral (*l.c.*), and assumed the long cilia in a latero-frontal position (*f.c.l.*) to be the true latero-frontal cilia. The arrangement of the cilia is so unusual that I have ventured to give a figure, although the material was preserved in alcohol for museum purposes and the fixation is imperfect. Examination of well-preserved material, and above all of the living gill is very desirable.

Pelseneer (1891, Pl. XIII, fig. 43) gave a figure of a transverse section of a filament of Trigonia pectinata in which no lateral cilia are labelled; it is possible that what he has named ciliated discs are the lateral, together with the latero-frontal, cilia. In Trigonia margaritacea, and also in Trigonia lamarki (see Ridewood, 1903), the ciliated discs are close to the interlamellar edge of the filament, and not as shown in Pelseneer's figure.

An interesting feature of the filaments of Trigonia margaritacea (alcohol preservation) is the presence of calcified rods (*c.r.*, Text-fig. 1 c), which were previously thought to occur only in Mülleria and the Unionidae (see p. 394).

In Xylophaga dorsalis the frontal cilia extend well round on to the lateral surfaces of the filaments, and the unciliated space between the latero-frontal and lateral cilia is rather wide. These peculiarities are not found in the allied Barnea parva.

(*b*) In Lamellibranchs with Micro-latero-frontal Cilia.

In these Lamellibranchs the arrangement of the various ciliary tracts appears to be more or less constant (see Text-fig. 3, A–E, p. 358), though the width of the frontal tract may vary, the cilia extending round on to the lateral faces; abfrontal cilia may, or may not, be present.

B. LATERO-FRONTAL CILIA AND PHYLOGENY.

DISCUSSION ON THE RELATIONSHIPS OF THE VARIOUS TYPES OF LATERO-FRONTAL CILIA.

From the work recorded in the foregoing pages it is now known that three variations of the latero-frontal tract of Lamellibranchs exist, namely: (1) eu-latero-frontal cilia, together with pro-latero-frontal cilia; (2) anomalous, together with para-latero-frontal cilia; and (3) micro-latero-frontal cilia. The second variation is known from one family only, the Ostreidae.

It is known that eu-latero-frontal and—though with less certainty—pro-latero-frontal cilia (see p. 356) are present in living Protobranchs, so that it is possible that the latero-frontal tract of the higher Lamellibranchs, with eu- and pro-latero-frontal cilia, may have been inherited from that order, and is not necessarily a new development. The presence of eu-latero-frontal cilia on gills, which, certainly in Nuculana and Nucula, are not used greatly in feeding, points to the antiquity of these cilia in the Protobranchia.

The relationship of the various types of latero-frontal cilia

is speculative whilst so little is known of the structure of the tenuous kinds of latero-frontal cilia (namely, the micro-, pro-, and para-latero-frontal cilia) and their cells: a detailed study of the histology of these was outside the scope of the present work, but a provisional comparison of the structure of the various types of latero-frontal cilia is given in Table I, p. 370. So far as is known the micro- and pro-latero-frontal cilia are closely comparable in structure; they are both of small size, are borne a number on a cell, and the cells and their nuclei are narrow and elongated in the direction of length of the filament. It is probable that these cilia are not composed of triangular plate-like elements, as are the eu-latero-frontal cilia of Mytilus and others, but of a few simple fibres. Whether, however, the micro- and pro-latero-frontal cilia are homologous, or only analogous, is not certain. The possibilities are that micro-latero-frontal cilia (a) are homologous with pro-latero-frontal cilia; (b) have given rise by specialization to eu- and to anomalous latero-frontal cilia; (c) are distinct structures, and so only analogous to pro-latero-frontal cilia; or (d) are reduced eu-latero-frontal cilia, though this is unlikely (see p. 387).

The anomalous latero-frontal cilia of the Ostreidae appear to be closely comparable in structure with eu-latero-frontal cilia, though of smaller size. They agree in that a single complex cilium arises from a cell, the cells are small, with the greater diameter at right angles to the length of the filament, and the arrangement of the ciliary rootlets appears to be similar in the two instances. However, it is extremely probable from a consideration of phylogenetic relationships, based on other characters (see p. 409), that the anomalous latero-frontal cilia of the Ostreidae are not homologous with eu-latero-frontal cilia, but that these cilia of similar structure have arisen independently in the Lamellibranchia, and that their similarity is due to convergence: one has not attained the size of the other.

In the Ostreidae not only are there anomalous but also para-latero-frontal cilia. It is therefore necessary to consider the origin of the two rows. One possibility is that micro-latero-frontal cilia, such as are found in other members of the group, have persisted as the para-latero-frontal cilia of the Ostreidae,

the anomalous arising as new structures not derived by modification from any pre-existing cilia. A second possibility is that the anomalous latero-frontal cilia have been derived from micro-latero-frontal cilia, perhaps by the approximation of these in pairs, and the formation of connecting membranes, the para-latero-frontal cilia being new structures, perhaps modified frontals. In this event there would have been progressive specialization of frontal cilia, the outermost of these being modified to form micro-latero-frontal cilia, and these again to form anomalous cilia, while a subsidiary row of latero-frontals (para-latero-frontal cilia) arose by modification of the now outer frontal cilia. An investigation into the composition of the latero-frontal tract in the Pteriacea to determine whether a second row of cilia is actually present in P i n n a, and may be in other members of the sub-order, might throw light on the question.

It has already been shown how the anomalous cilia of O s t r e a could have been derived from the type of cilia of the main row in P i n n a (see p. 362), and if two rows should occur not only in P i n n a, but in other members of the Pteriacea, it seems very possible that the anomalous latero-frontal cilia have originated in some such manner.

The appearance of latero-frontal cilia in the Lamellibranchia, whether evolved independently in the different lineages, or as modifications of one ancestral type must be considered. To the lateral and frontal cilia inherited probably from ancestors common to both Gastropods and Lamellibranchs (Pelseneer, 1891) have been added latero-frontal cilia. The latero-frontal tracts, however, are not all of the same type, and the possibilities are either that the variations have been derived from one original type, or that the various branches of Lamellibranchs have independently evolved the same straining device, that is, the same sort of latero-frontal cilia on each independent line of evolution. The evolutionary tendency has been directed in this event toward the attainment of complex cilia composed of triangular plates, namely eu- and anomalous latero-frontal cilia.

Four suggestions may be made: (*a*) The ancestral Lamellibranchs may have had a single row of micro-latero-frontal cilia

on each side of the frontal tract, probably arising by modifica-
tion of the outer frontal cilia. This constitution of the straining
apparatus persisted in most members of a branch represented
to-day by the Arcidae, Anomiidae, and Pseudolamellibranchia,
but in certain members there was an attempt at the production
of a more efficient apparatus by the addition of larger cilia,
possibly in Pinna, and certainly in the anomalous cilia of the
Ostreidae, the highest, though by no means the most recent,
member of the group. In another branch, or branches, the
addition of large complex cilia to the original micro-latero-
frontal tract took place early, such branch or branches being
represented by the Protobranchia, Mytilidae, Trigoniidae, and
Eulamellibranchia, in which the straining apparatus consists
of a row of pro- (= micro ?) and a row of eu-latero-frontal cilia
on each side of the frontal tract. There would thus have been
parallel evolution of complex cilia, eu-latero-frontal cilia in one
branch or branches, and anomalous in another. As suggested
previously, when two rows are present, the micro-latero-frontal
cilia may have persisted as para- and pro-latero-frontals (that
is, all are homologous), the anomalous and eu-latero-frontal cilia
being new structures, not derived from any pre-existing cilia;
or the two rows may have arisen by progressive modification
of the outer frontal cilia, in which case the eu- and anomalous
latero-frontal cilia would have arisen by specialization of micro-
latero-frontal cilia.

(b) Secondly, the micro-latero-frontal cilia type and the eu-,
together with pro-latero-frontal cilia type, may have been inde-
pendently evolved from forms in which no latero-frontal cilia
were present, the micro- and pro-latero-frontals being ana-
logous.

(c) Thirdly, the original type of latero-frontal tract may have
been eu-, together with pro-latero-frontal cilia, this type per-
sisting in the branch or branches represented by the Proto-
branchia, Mytilidae, Trigoniidae, and Eulamellibranchia. The
loss of eu-latero-frontal cilia would then have to be postulated
in the group represented by the Arcidae, Anomiidae, and
Pseudolamellibranchia, with a fairly successful attempt at the
regaining of complex cilia in the anomalous latero-frontal cilia

of the Ostreidae. This loss and then recovery of complex cilia is unlikely. If this has occurred then the micro- would be homologous with the pro-, but not necessarily with the para-latero-frontal cilia.

(d) Fourthly, the original type of tract may have been eu-latero-frontal cilia alone. To these pro-latero-frontal cilia were added in Protobranchia (probably), Mytilidae, Trigoniidae (possibly), and Eulamellibranchia. It would then be necessary to postulate the reduction of eu- to micro-latero-frontal cilia in the Arcidae, Anomiidae, and Pseudolamellibranchia, and either their enlargement to anomalous cilia with the addition of para-latero-frontal cilia in the Ostreidae, or the persistence of micro- as para-, and the addition of anomalous latero-frontal cilia.

Of these four possibilities (c) and (d) seem the most improbable.

The evolution of straining cilia has apparently occurred only in the Lamellibranchia, no other group of ciliary feeders, so far as is known, having acquired this refinement of the ciliary method of feeding.

Essential ciliated tracts of the food-collecting mechanism of the gills are the lateral, as current producing, and the frontal cilia, for conveying material, these being found in both Gastropods (Streptoneura) and Lamellibranchs: analogous tracts occur in several groups of ciliary feeders outside the Mollusca. The latero-frontal or straining cilia increase the efficiency of the ciliary method of feeding, but apparently are not essential. Latero-frontal cilia have not been described in any Gastropod, so that it may be taken for granted that eu-latero-frontals are absent. The only Gastropods that were examined for micro-latero-frontal cilia were the Pectinibranch Crepidula fornicata and the Rhipidoglossate Aspidobranch Calliostoma zizyphinum, and in these species I was unable to find them: Crepidula is a ciliary feeder (Orton, 1912), while Calliostoma possibly does not consume the material collected by the gills. Though this is very slight evidence indeed, it is perhaps safe to presume that latero-frontal cilia of any kind are absent in Gastropods, and that these ciliary structures have arisen in, and are characteristic of the Lamellibranchia. It is possible

that the appearance of these structures in the Lamellibranchia may be connected in some way with the loss of the radula; it is hoped to go into this in a later paper.

THE TAXONOMIC VALUE OF THE LATERO-FRONTAL CILIATED TRACTS.

Gill structure as a means of classification has been criticized in that it is liable to progressive modification, and thus reliance on it tends to produce 'horizontal' rather than 'vertical' divisions (Douvillé, 1912 a; Davies, 1933). The composition of the latero-frontal ciliated tracts seems to be a more or less stable character, in fact, except for the Ostreidae, it was found that bivalves (117 species representing 51 families investigated) have one or other of two types, namely: (a) eu-, together with pro-latero-frontal cilia; or (b) micro-latero-frontal cilia alone. With the exception of the Ostreidae, no bivalve has been found in which the latero-frontal ciliated tracts cannot be easily determined to belong to one or other of these types. This character is therefore not open to the above-mentioned objection. But while the composition of the latero-frontal ciliated tracts may be used to separate a large group with micro-latero-frontal cilia from a much larger group with a row of eu- and a row of pro-latero-frontal cilia on each side of the frontal surface, it is useless, so far as my knowledge goes, for the determination of smaller divisions: this, however, may not prove to be so in the group characterized by the possession of micro-latero-frontal cilia when more is known of the structure of these cilia.

In the following discussion it is claimed, or assumed, that the character of the latero-frontal cilia affords a real clue to the broad affinities of bivalves, and offers a test of existing classifications.

REVIEW OF PELSENEER'S AND RIDEWOOD'S CLASSIFICATIONS OF THE LAMELLIBRANCHIA.

The two classifications of the Lamellibranchia best known to zoologists are perhaps those of Pelseneer (1906) and Ridewood (1903), based largely on gill structure; that of Pelseneer being the more widely accepted. Pelseneer, in 1911, returned

to his older classifications of 1889, 1891, and 1892 retaining the
Order Pseudolamellibranchia, including the Aviculacea with the
Pinnidae and Ostreidae, and the Pectinacea with the Limidae.

The two classifications are given briefly on p. 390, in relation
to, and so far as they affect the present work. The composition
of the latero-frontal ciliated tracts in the various families is
indicated, so that it may be seen at a glance how the different
types are dispersed through the sub-orders and orders of these
classifications.

In Ridewood's classification, forms previously considered allied
are widely separated, and dissimilar forms associated. He
(p. 184), himself, stated that 'It is not claimed that the scheme
of classification set forth in the following pages represents the
genetic affinities of the forms included; but while disinclined
to inflict upon a long-suffering world of zoologists a new classi-
fication of the Lamellibranchia in which I myself have no great
confidence, I have, for reasons similar to those stated in a
previous paper, arrived at the conclusion that, for purposes of
ready reference, a key to the species examined based on the
particular feature under consideration is not only justifiable,
but even useful.'

Examining Ridewood's classification in some detail we find
that in the Protobranchia the two families, Nuculidae (in which
he included the Nuculanidae) and Solenomyidae, have eu-
latero-frontal cilia, most probably also with pro-latero-frontal
cilia at least in the Nuculidae (see p. 356).

His order Eleutherorhabda can be shown to be not less than
diphyletic, some families possessing eu- together with pro-
latero-frontal cilia, and others micro-latero-frontal cilia only.
He defined the sub-orders as follows: Dimyacea; gill lamellae
flat and homorhabdic, with no ascending filaments: Mytilacea;
gill lamellae flat and homorhabdic, with ascending filaments:
Pectinacea; gill lamellae plicate and heterorhabdic, with ascend-
ing filaments.

In the Dimyacea he placed Dimya argentea and
Anomia aculeata (=Heteranomia squamula). Het-
eranomia has micro-latero-frontal cilia: whatever the form
of the latero-frontal cilia of Dimya—which I have not had

RIDEWOOD'S AND PELSENEER'S CLASSIFICATIONS OF THE LAMELLIBRANCHIA.

Ridewood (1903).	Pelseneer (1906, modified 1911).
Order 1. PROTOBRANCHIA. Nuculidae,[2] Solenomyidae.[2]	Order 1. PROTOBRANCHIA. Solenomyidae,[2] Nuculidae,[2] Ledidae.[2]
Order 2. ELEUTHERORHABDA. Sub-order 1. Dimyacea: Dimya,[5] Anomia aculeata (= Heteranomia squamula),[4] 2. Mytilacea: Anomiidae (excluding Anomia aculeata),[4] Arcidae,[4] Trigoniidae,[1] Mytilidae,[1] Melinidae,[4] Amussiidae.[4] 3. Pectinacea: Spondylidae,[4] Pectinidae,[4] Aviculidae.[4]	Order 2. FILIBRANCHIA. Sub-order 1. Anomiacea: Anomiidae.[4] 2. Arcacea: Arcidae,[4] Pectunculidae,[4] Philobryidae,[5] Trigoniidae.[1] 3. Mytilacea: Mytilidae.[1] 4. Dimyacea: Dimyidae.[5]
Order 3. SYNAPTORHABDA. Sub-order 1. Ostreacea: Pinnidae,[4] Limidae,[4] Ostreidae.[3] 2. Submytilacea.[1] 3. Tellinacea.[1] 4. Veneracea.[1] 5. Cardiacea.[1] 6. Myacea.[1] 7. Pholadacea.[1] 8. Anatinacea.[1] 9. Poromyacea.[5]	Order 3. PSEUDOLAMELLIBRANCHIA. Sub-order 1. Aviculacea: Aviculidae,[4] Vulsellidae,[4] Pernidae,[4] Pinnidae,[4] Ostreidae.[3] 2. Pectinacea: Pectinidae,[4] Amussiidae,[4] Spondylidae,[4] Limidae.[4] Order 4. EULAMELLIBRANCHIA. Sub-order 1. Submytilacea.[1] 2. Tellinacea.[1] 3. Veneracea.[1] 4. Cardiacea.[1] 5. Chamacea.[5] 6. Myacea.[1] 7. Adesmacea.[1] 8. Anatinacea.[1] Order 5. SEPTIBRANCHIA.[5]

[1] Eu- together with pro-latero-frontal cilia (but see p. 368).   [2] Eu- together with para-latero-frontal cilia.   [3] Anomalous together with pro-latero-frontal cilia apparently present (see p. 356).   [4] Micro-latero-frontal cilia.   [5] No members examined.

an opportunity of examining—there seems no justification for separating Heteranomia from the rest of the Anomiidae.

Of the six families placed in the Mytilacea, the Anomiidae, Arcidae, and Melinidae have micro-latero-frontal cilia, and their presence is inferred in the Amussiidae owing to the close similarity of the gills of Amussium pleuronectes to those of the Pectinidae in which these cilia occur, although latero-frontal cilia could not be distinguished because of poor preservation (Atkins, 1938).

In his family Melinidae Ridewood placed the genera Melina (=Perna =Isognomon) and Malleus. Of these two genera, micro-latero-frontal cilia have been distinguished in Malleus albus (*mi-l-f.c.*, Text-fig. 3 B) (see, however, p. 363): the gills of Isognomon alata and Isognomon isognomon were not sufficiently well preserved to distinguish micro-latero-frontal cilia, but eu-latero-frontals were almost certainly absent. It might here be observed that Pelseneer in his classification of 1906 placed Malleus in the Aviculidae, but followed Ridewood in assigning Perna (Pernidae) to the Mytilacea: in 1911 he replaced this latter family in the Aviculacea.

In the Amussiidae Ridewood included Plicatula australis, which Pelseneer (1906) placed in the Spondylidae, and Watson (1930) concluded should either be a distinct family in the Pectinacea, or the Pectinidae should be divided into not less than four sub-families—the Amussiinae, Plicatulinae, Pectininae, and Spondylinae. I have been unable to obtain material of Plicatula.

There can be no doubt but that Ridewood was wrong in including the Amussiidae in the Mytilacea even on a consideration of the form of the gill. It has been shown in a previous paper (Atkins, 1938) that the gills of Amussium pleuronectes are plicate and heterorhabdic, and in fact very closely resemble those of certain of the Pectinidae, and unless it is to be separated from members of the family with flat and homorhabdic gills (Amussium dalli, Amussium meridionale, Amussium lucidum), then the natural position of the Amussiidae, from a consideration of general anatomy, is near the Pectinidae in the sub-order Pectinacea.

The Amussiidae are not alone among the Eleutherorhabda in containing some members with flat and homorhabdic, and others with plicate and heterorhabdic gills. In the Pectinidae a species, Pecten groenlandicus, with flat and homorhabdic gills is known (Noman, 1882). In fact in this family there is considerable variation in the structure of the gills (see Text-fig. 12, p. 414). The simplest is Pecten groenlandicus with flat and homorhabdic lamellae, lacking interlamellar septa, and with a single row of ciliated discs, those at the lower edge of the demibranch; most known species appear to have plicate and heterorhabdic lamellae, with interlamellar septa, and with a number of ciliary interfilamentar junctions as in Pecten maximus, Pecten irradians, Chlamys opercularis, Chlamys distorta, Chlamys tigerina, and Chlamys vitrea; the most highly developed would seem to be Pecten tenuicostatus Mighels (= Pecten grandis Solander) in which organic interfilamentar junctions occur, though ciliary ones are present near the free margin of the demibranch (Drew, 1906, 1907 a). In the Pteriidae, Stempellaria magellanica (see Clasing, 1921) and Malleus albus,[1] have flat and homorhabdic gills, while Pteria and Pinctada have plicate and heterorhabdic gills. In the Vulsellidae, within the same genus, one species Vulsella rugosa, has flat gills, and another, Vulsella lingulata, plicate gills, (see Pelseneer, 1911). It will thus be seen that the difference between flat and plicate gills does not warrant the separation of related species and genera, as Pelseneer recognized in 1911.

Of the sub-order Mytilacea there now remains the Mytilidae and Trigoniidae to be considered. These two families alone of Ridewood's sub-order have eu-latero-frontal cilia, together with pro-latero-frontals in the Mytilidae (the filaments of Trigonia margaritacea were not well enough preserved to make identification of pro-latero-frontals possible) and most probably

[1] There seems some doubt as to the family in which Malleus should be placed. Jackson (1890) considered it as closely allied to Avicula, and provisionally placed Vulsella (Vulsellidae), and Malleus on the same branch from Avicula: Pelseneer (1906, 1911) placed Malleus in the Aviculidae itself.

belong to a distinct phylogenetic group, or groups, from that of the rest of the families in the sub-order. It seems very improbable that there is any close relationship between the Mytilidae and the Trigoniidae, for the ciliation of the filaments is markedly dissimilar (see p. 381; Text-fig. 1, A and C, p. 351). The position of the Trigoniidae will be discussed later (p. 394).

Little need be said of the three families, Spondylidae, Pectinidae, and Aviculidae, placed by Ridewood in the sub-order Pectinacea: all three possess micro-latero-frontal cilia.

Ridewood (1903, p. 181) stated his reasons for the separation of forms previously classed in the Pectinacea and their inclusion in the Mytilacea as follows: 'Of the genera of the Pseudolamellibranchia which it is now proposed to associate with the Filibranchia under the title Eleutherorhabda, some have well-developed principal filaments and plicate lamellae (e.g. A v i - c u l a,   M e l e a g r i n a,   P e c t e n,   S p o n d y l u s), whereas others have flat lamellae and filaments undifferentiated (e. g. P l i c a t u l a,   M a l l e u s,   M e l i n a,   A m u s s i u m,   and D i m y a). So sharply marked off are these first four genera from all the rest of the Eleutherorhabda by reason of their strongly differentiated principal filaments and their plicate lamellae, that it is well to let them constitute a sub-order by themselves.' It is difficult to follow why Ridewood should have ascribed such importance to the difference between flat homorhabdic, and plicate heterorhabdic lamellae in the Eleutherorhabda, while considering (p. 161) that in the Synaptorhabda such a difference was of not more than specific, or at most subgeneric, value; and after drawing attention to the fact that principal filaments are ontogenetically a secondary differentiation, in P e c t e n for example (p. 162). Pelseneer in 1911 justly criticized Ridewood's use of this character, and also his use of the structure of the interfilamentar junctions, whether ciliary or vascular, to divide the Pseudolamellibranchia among the Eleutherorhabda and Synaptorhabda.

It is necessary to consider Ridewood's sub-order Ostreacea, which he placed in the order Synaptorhabda along with seven sub-orders now known to possess eu- together with pro-latero-frontal

cilia, and the sub-order, Poromyacea, which included bivalves generally classed in a separate order Septibranchia. Of the three families of the sub-order Ostreacea, the Limidae and Pinnidae possess micro-latero-frontal cilia, the Pinnidae possibly with an additional row (see p. 363), while the Ostreidae have anomalous, together with para-latero-frontal cilia. Thus the composition of the latero-frontal tract indicates that the Synaptorhabda, as the Eleutherorhabda, are at least diphyletic, and the Ostreacea should be removed from the Synaptorhabda as Pelseneer contended in 1911.

We have now to see how Pelseneer's classification is affected by a consideration of the composition of the latero-frontal ciliated tracts, and it may be said at once that it is only in the Filibranchia and Pseudolamellibranchia that any change is suggested.

In the Protobranchia the three families Solenomyidae, Nuculidae, and Ledidae (= Nuculanidae) all have eu-latero-frontal cilia, most probably with pro-latero-frontal cilia also, as found in the Nuculidae (see p. 356).

His order Filibranchia, as Ridewood's order Eleutherorhabda, is almost certainly diphyletic, if not triphyletic. The Anomiidae, Arcidae, and Pectunculidae have micro-latero-frontal cilia; the Trigoniidae and Mytilidae eu-latero-frontal cilia, together with pro-latero-frontals certainly in the Mytilidae. Pelseneer (1889, 1891, 1892, 1906) derived the Trigoniidae from the Arcidae and placed the two families together in the sub-order Arcacea. The difference in the composition of the latero-frontal tracts, as well as the arrangement of the various ciliary tracts of the filaments, in the two families, indicate that they belong to distinct phylogenetic groups, though at about the same level of gill and mantle evolution. From a consideration of the different arrangement of the ciliary tracts in the Trigoniidae and Mytilidae it is unlikely, as previously mentioned, that these two families are at all closely related. The position of the Trigoniidae is of much interest, and in this connexion a peculiarity of the filaments of Trigonia margaritacea may be mentioned here; that is, the presence of calcified rods[1] embedded in the chitinous sup-

[1] These were obvious in entire filaments of alcohol-preserved material,

porting structure (*c.r.*) (Text-fig. 1 c, p. 351). The presence of these rods in T r i g o n i a is curious, as, according to Ridewood (1903, p. 168), calcified rods are peculiar to the filaments of the Unionidae and M ü l l e r i a. They have been described in A n o d o n t a, U n i o, M ü l l e r i a, and M o n o c o n d y l a e a: from the appearance of sections of the filaments of M u t e l a b o u r g u i g n a t i (Text-fig. 6 B, p. 371) it is evident that they are also present in that species. Calcified rods are thus characteristic of a number of the Naiadacea: they appear, however, to be absent in A e t h e r i a; Ridewood (p. 230) did not mention them in his description of the gills of A e t h e r i a p l u m b e a, and there is no indication of them in the filaments of A e t h e r i a e l l i p t i c a. Ridewood may have overlooked their presence in T r i g o n i a l a m a r k i, unless this is a character which varies in related species.

Neumayr (see Douvillé, 1912 a) concluded from a study of the hinge that the Unionidae and Trigoniidae were closely related. Douvillé (1912 a, p. 443), however, has pointed out that the similarity between the hinges of U n i o and T r i g o n i a is delusive, for the tooth of T r i g o n i a is not originally double as that of the Unios, but is derived by division from a primitively simple tooth. Douvillé, himself, derived the Trigoniidae from the Preheterodonts by way of the Myophoriidae, and considered that they gave rise to no higher forms. Prashad's (1931, p. 48) objection to the association of the Unionidae (or super-family of the Naiadacea) and the Trigoniidae in the same order, Schizodonta, is based on the difference in the structure of the gills in the two families, the former being eulamellibranchiate and the latter filibranchiate. The difference as between the two types of gills seems to me not of great importance in view of the occurrence of both ciliary and organic

owing to their having been fractured at intervals. The two main rods occur toward the interlamellar ends of the filaments and are continuous throughout the length of the filament. Two other, but much smaller, rods are situated beneath the lateral ciliated cells; they appear to be only intermittently present. Material prepared for sectioning was soaked for a short time in weak hydrochloric acid alcohol; the part of the rods remaining after the treatment stains intensely with iron haematoxylin, as indicated in Text-fig. 1 c (p. 351).

interfilamentar junctions in the same individual in Pecten tenuicostatus, of compound ciliary and organic junctions in certain species of Pteriidae, Limidae, and Pinnidae (see p. 406), and of the apparent ease with which extensive interfilamentar and interlamellar junctions are produced in abnormal gills of the filibranchiate species, Mytilus edulis (Atkins, 1930). A greater difficulty is the arrangement of the ciliated tracts of the filaments, for this is likely to be a more stable character, few variations being known (see p. 380). Though the three families of the Naiadacea, the Unionidae, Aetheriidae, and Mutelidae, have eu-latero-frontal cilia as have the Trigoniidae, yet the arrangement of the various tracts of cilia is that common to the Mytilidae and the Eulamellibranchs possessing eu-latero-frontal cilia (Text-figs. 1 A, B, p. 351; 6, p. 371), while the arrangement in Trigonia margaritacea is entirely different (Text-fig. 1 c). Although this would not be an insuperable difficulty in the way of the derivation of the Unionidae from the Trigoniidae, or from a common ancestor, it would seem to indicate that the two families are not closely related, in this agreeing with the conclusion reached by Douvillé. The calcified rods of the two families may quite possibly have been developed independently. It must not be overlooked, however, that there is nearly as great a difference in the arrangement of the ciliary tracts in two families of Protobranchs, the Nuculidae, and Nuculanidae, which at one time were classed as one family, as in the Trigoniidae and Unionidae.

In Pelseneer's order Pseudolamellibranchia are included a number of families with micro-latero-frontal cilia, and one, the Ostreidae, with anomalous together with para-latero-frontal cilia. My material representing the families Vulsellidae and Pernidae (= Isognomonidae) was not sufficiently well preserved to allow of the identification of micro-latero-frontal cilia, but eu-latero-frontals are almost certainly absent; it may perhaps be assumed that the former kind of cilia are present (see p. 376). Micro-latero-frontal cilia were identified in Malleus albus, but there seems some uncertainty as to whether Malleus is a Vulsellid or a Pteriid (see p. 392). The presence of micro-latero-frontal cilia is assumed in the Amussiidae, owing to the

close similarity of the gills of Amussium pleuronectes to those of the Pectinidae (Atkins, 1938).

The families of the Pseudolamellibranchia are closely related, and, as will be seen later, in all probability form with the Arcidae and Anomiidae a monophyletic group.

In his classification of 1906 Pelseneer placed the Aviculidae, Vulsellidae, Amussiidae, Spondylidae, and Pectinidae (and certain extinct families) together in the sub-order Pectinacea. The last three families mentioned are more closely related to one another than they are to the first two, and this he recognized in 1911 in creating a sub-order Aviculacea for the first two families together with the Isognomonidae and Pinnidae, and with the Ostreidae as an offshoot, and placing the last three together with the Limidae in the Pectinacea.

Pelseneer's view of the close relationship of the families of the Pectinacea is borne out by work on the gills. Apart from the common possession of micro-latero-frontal cilia the members of the Amussiidae, Pectinidae, Spondylidae, and Limidae investigated (which were all forms with plicate and heterorhabdic lamellae) agree very closely in the structure of the gills. They have: (1) a suspensory membrane to the gill; (2) a similar arrangement of the muscles of the gill axis; (3) a similar arrangement of the horizontal muscles of the principal filaments; (4) few or no vertical muscles in the lamellae; (5) respiratory expansions on the principal filaments; and (6) chitinous supporting structure of the principal filaments of similar form in all except the Limidae. In the forms with flat and homorhabdic gills, for example Amussium dalli, Amussium lucidum, Amussium meridionale, and Plicatula australis, respiratory expansions are apparently absent.

Pelseneer's order Eulamellibranchia contains only families, which, so far as my work has gone, possess eu- together with pro-latero-frontal cilia. Palaeontologists, however, consider that this order is not a monophyletic one, and Douvillé (1912a) has divided it into two groups, a 'normal' and a 'burrowing' branch. The possession of the same type of latero-frontal tract by both groups may be explained by their probable origin, the 'normal' branch from a nuculoid form, and the 'burrowing' branch from

a form of which Solenomya may be considered representative (Davies, 1933): both Nucula and Solenomya have eu-latero-frontal cilia (Text-fig. 7, p. 372), and, as it has been seen (p. 356), Nucula, if not Solenomya, probably has pro-latero-frontal cilia as well. In the phylogenetic division of the Eulamellibranchia the form of the latero-frontal cilia is therefore apparently of no assistance. It is curious that a certain type of pattern of the lateral ciliated cells is common among both 'normal' and 'burrowing' groups of Lamellibranchs (Atkins, 1938 a). Differences in the detailed arrangement of these cells would seem to be of doubtful utility, and the same pattern occurring in different bivalves might be expected to indicate relationship. This character may repay a fuller investigation than I was able to give to it (1938 a). It is perhaps possible that the 'normal' and 'burrowing' groups of Lamellibranchs are not as widely separated as suggested by Douvillé's table (1912 a, p. 466).

Relationship of Lamellibranchs with Micro-latero-frontal Cilia.

Bivalves with micro-latero-frontal cilia, those previously considered as lacking such cilia, are not scattered haphazard through the Lamellibranchia, but are found in families which are more or less closely related (see Text-fig. 9), forming, except for my inclusion of the Arcidae, a group designated 'the Aviculidae and their allies' by Jackson (1890). This is the same group as Douvillé's (1912 a) 'sedentary' branch, except for his inclusion of the Mytilidae. That a group established by palaeontologists almost entirely on shell characters should be related also by the condition of a certain tract of cilia on the gill filaments is most interesting.

Jackson (1890, p. 362) found the Aviculidae and their allies to be linked in one great group by characters of anatomy and shell structure which connect the several members, and to be characterized 'by possessing prodissoconchs of homogeneous laminar structure, but not prismatic, and with umbos directed posteriorly'.[1] The suc-

[1] Professor A. Morley Davies informs me by letter that '"umbos

ceeding dissoconch has 'an external layer of prismatic cellular tissue which is more or less developed but exists at least in the early nepionic stages of one valve' (1890, p. 377).

Douvillé (1912 a, p. 460) characterized his 'sedentary' branch as 'essentiellement byssifères'. He considered that fixation by the byssus has impressed certain characters on the group which tended to persist through various changes in the mode of life. This method of fixation may be accomplished in very different ways according to the length of the byssus. When it is so short that fixation is practically by the foot, as in Arca, it results in an inequilateral shell, the posterior region, where the exhalent and main inhalent apertures are situated, being more developed than the anterior, but with little or no difference in the size of the adductor muscles. As the byssus lengthens pressure is exerted by the foot on the anterior adductor, and as the pressure increases this muscle diminishes in size, as in the Pinnidae, and finally disappears (many forms of the group). The reduction of the anterior adductor is accompanied by that of the anterior region generally.

Byssal fixation and cementation, however, are not confined to the 'sedentary' branch, and in two families at least, the Aetheriidae and Tridacnidae, may be accompanied by the monomyarian condition. According to Yonge (1936), however, in the Tridacnidae this condition was brought about in an entirely different way—by rotation of the mantle and shell round the visceral mass—from that in the 'sedentary' branch. Douvillé, of course, included the Mytilidae, in which byssal fixation has resulted in reduction and disappearance of the anterior adductor muscle in some species, in his 'sedentary' group: the position of the Mytilidae will be discussed later.

The group (Arcidae, Anomiidae, Pteriacea with Pinnidae, Ostreidae, Pectinacea with Limidae) characterized by the possession of micro-latero-frontal cilia would appear in all probability to be a monophyletic one (Text-fig. 9). As previously

directed posteriorly" is not, as it might seem at first sight, an embryonic or juvenile character, but is an effect of later unequal growth. Possibly its general presence in "the Aviculidae and their allies" may be connected with the reduction of the anterior region of the shell'.

Text-fig. 9.

Geological occurrence and phylogenetic inter-relationships of the group of Lamellibranchs characterized by the possession of micro-latero-frontal cilia (only families and genera examined included). Geological occurrence obtained largely from Dall (in Zittel, 1913); phylogenetic relationships largely from Jackson, 1890. (Arcidae is used in a wide sense to include Glycymeris and allied genera, and Parallelodon and allied genera).

stated, it differs from Jackson's group in the inclusion of the Arcidae, but this ancient family appears from palaeontological evidence to be connected with the Pteriacea, for according to Arkell (1930, pp. 306–7) it can scarcely be doubted that C y p r i-c a r d i t e s (= P a l a e a r c a), which ranges from the Ordovician to the Devonian, and P a r a l l e l o d o n, a genus of Arcidae, were descended from a common stock. C y p r i c a r d i t e s was placed in the Arcacea by Dall (in Zittel, Eastman edition, 1913, vol. i reprinted in 1927 and 1937), but Zittel (German edition, 1924, see Arkell) classed it with the Ambonychiidae, a family of the Pteriacea. Douvillé (1912 a) associated it with the Pterineidae. He held that the ancient forms of the Arcidae approach much more to the Pterineidae than to the Nuculidae, but thought that the Arcidae really constitute a branch parallel to that of the Aviculidae (= Pteriidae), but fixed only by the foot, and in consequence with a much less great reduction of the anterior adductor muscle, and of the anterior portion of the animal generally. In the living genus L i m o p s i s (sometimes classed with the Arcidae, sometimes in a separate family, and which according to Pelseneer (1911, pp. 8, 9) should, with P e c t u n c u l u s (= G l y c y m e r i s), be placed in a family Pectunculidae, apart from the Arcidae), however, the anterior adductor muscle is frequently much reduced, and in an allied family, the Philobryidae, is wanting (Pelseneer, 1906, p. 258).

Palaeontologists differ as to the position of the Mytilidae. This family was included by Douvillé in his 'sedentary' branch, but omitted by Jackson from 'the Aviculidae and their allies'. Douvillé (1912 a, p. 460) distinguished two great groups in the 'sedentary' branch: (1) the Pterineidae with straight hinge, and amphidetic ligament, at first simple and inserted on a more or less developed area; and (2) the Mytilidae with curved hinge, opisthodetic ligament, remaining marginal, and without an area. Jackson (1890, p. 364), on the other hand, stated that 'The striking differences in the prodissoconch and nepionic stages of the Mytilidae and Aviculidae are sufficient, I think, to separate these groups, and the Mytilidae should be put in a group distinct from the Aviculidae and their allies, which I have shown are all bound together by important features as one

group'. His conclusions are supported by the difference in the composition of the latero-frontal tracts in the Mytilidae and in the Pteriidae and their allies (see pp. 368–380).

Comparing the group with micro-latero-frontal cilia with Pelseneer's classification of 1911 it will be seen that this group corresponds to his order Pseudolamellibranchia (see p. 390) together with the Arcidae and Anomiidae, which last two families he associated with the Mytilidae and Trigoniidae in the Filibranchia. As previously stated, the Arcidae and Anomiidae have a type of latero-frontal tract quite distinct from that of the other two families of the Filibranchia, with which they appear to have no close relationship.

Pelseneer abandoned the order Pseudolamellibranchia in 1906, but in 1911 (p. 119) he stated: 'je suis ramené à mon idée ancienne et première qu'il faut reconstituer un groupe entre les Filibranches et les Eulamellibranches—groupe représentant un stade phylogénétique postérieur au premier et formant une branche, globalement moins spécialisée que le second et orientée dans une autre direction.' He (1911, pp. 120–1) defined it as follows: (1) the gills have ciliary or cellular interfilamentar junctions (or both) and are free or attached to the mantle by ciliary junctions, but (2) the pallial lobes are without suture (whilst all Eulamellibranchs have always non-ciliary and vascular interfilamentar and interlamellar junctions, and always one or several pallial sutures; (3) the auricles inter-communicate; (4) the presence of abdominal sense organs[1] on the posterior adductor muscle; (5) the anterior adductor muscle is wanting; (6) the byssus is normally well developed.

Pelseneer's order Pseudolamellibranchia is not sufficiently inclusive, for the present work indicates that the Arcidae and Anomiidae are related to the families forming that order. Palaeontological evidence for the inclusion of the Arcidae has been briefly given (p. 399); it remains to consider the Anomiidae.

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[1] Paired abdominal sense organs are present on, or near, the posterior adductor muscle in the Arcidae, whilst a single organ is present on the right side only in Placuna placenta (Anomiidae), as in Pecten (Willey, 1911, pp. 158–61). Paired abdominal sense organs also occur in the Trigoniidae (Pelseneer, 1906) and in the Mytilidae (Field, 1922).

The present research certainly does not support Pelseneer's (1911, p. 16) contention—in opposition to Jackson, Bernard, Rice, and Stenta—that the affinities of Anomia are with the Filibranchia and especially with the Mytilidae. Although the form of the latero-frontal cilia cannot help in solving the problem of the immediate ancestry of the Anomiidae, whether they are derived from the Pectens, as Jackson supposed, or from some other member of the group, it at least indicates that they are members of 'the Aviculidae and their allies'. Jackson (1890, p. 362) thought it possible that Anomia was derived from the Amussium or Hemipecten group of the Pectens, as these resemble Anomia in having thin nacreous shells. According to Douvillé (1912 a) the nacreous type of shell precedes the porcelaneous in evolution, and forms derived from porcelaneous types are always porcelaneous; it therefore seems that Anomia, whatever its ancestry may prove to be, must have been derived from forms with nacreous shells. The Anomiidae are known certainly from the Jurassic: there is a record of Anomia in the Devonian, but this occurrence seems doubtful as there is no other record before the Jurassic.

To summarize the various views considered here: Jackson saw in 'the Aviculidae and their allies' a natural group from which the Mytilidae are clearly separated; he did not include the Arcidae. For Douvillé his 'sedentary' branch is 'essentiellement byssifères'. It contains not only the forms included in Jackson's group, but also the Arcidae (which he considered really constitute a branch parallel to that of the Pteriidae) and the Mytilidae. He distinguished two great groups in the 'sedentary' branch, the Pterineidae and the Mytilidae, separated by certain characters of the hinge. For Pelseneer his order Pseudolamellibranchia is a group between the Filibranchia and the Eulamellibranchia, though not ancestral to the latter, its members connected by a number of characters in common. It corresponds to Jackson's group less the Anomiidae, which family Pelseneer contended has affinities, not with the Pseudolamellibranchia, but with the Mytilidae.

The group characterized by the possession of micro-laterofrontal cilia corresponds to Jackson's group of 'the Aviculidae

and their allies' with the addition of the Arcidae; to Douvillé's 'sedentary' branch less the Mytilidae; and to Pelseneer's Pseudolamellibranchia with the addition of the Arcidae and Anomiidae. The sub-orders and families in the group are then (so far as my material goes) as follows:

Arcacea: Arcidae.

Anomiacea: Anomiidae.

Pteriacea: Pteriidae, Isognomonidae, Vulsellidae, Pinnidae.

Pectinacea: Pectinidae, Amussiidae, Spondylidae, Limidae.

Ostreacea: Ostreidae.

The geological occurrence and phylogenetic interrelationships of the group are shown in Text-fig. 9, p. 400.

DISCUSSION ON THE ORIGIN OF THE GROUP CHARACTERIZED BY THE POSSESSION OF MICRO-LATERO-FRONTAL CILIA.

Eu-latero-frontal cilia are present in living representatives of all three existing families of the Protobranchia, together with pro-latero-frontal cilia at least in Nucula. The presence of these large straining cilia on gills which, certainly in Nucula and Nuculana, play only a subsidiary part in feeding, points to the antiquity of eu-latero-frontal cilia, and incidentally suggests that the gills of ancient Protobranchs may perhaps have played a greater part in feeding than do those of most of their modern representatives. This has already been suggested for the Nuculanidae on the evidence of the complicated frontal currents present on the small gills of Nuculana minuta (Atkins, 1936). It may very well be that in the Protobranchia, suspension feeding (as in Solenomya) is more primitive than deposit feeding (as in Nucula and Nuculana).

It has been generally considered that the Nuculidae,[1] or

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[1] Schenck (1934) in his paper on 'Classification of Nuculid Pelecypods' though not doubting that the family Nuculidae has Devonian representatives, yet insisted that he had seen no Nucula, sensu stricto, in rocks of Palaeozoic age, the Palaeozoic specimens he had studied not being closely related to the type species of Nucula, s.s.

nuculoid forms, are the stock from which sprang the higher Lamellibranchs (Jackson, 1890; Pelseneer, 1891, 1911; Rice, 1897, &c.), with the exception of Douvillé's 'burrowing' branch, or Desmodonts, of which Davies (1933) suggested S o l e n o m y a as representative of the ancestral form. If this be so, and if eu-together with pro-latero-frontal cilia were present in primitive Nuculidae, as they are in their living representatives in the genus N u c u l a, then it would seem to follow that from the Nuculidae have arisen at least two lines, one in which this type of tract has persisted (Mytilidae, Trigoniidae, Eulamellibranchia) and the other in which eu-latero-frontal cilia have been lost (Arcidae, Anomiidae, Pseudolamellibranchia), excluding the possibility of their reduction. It seems very improbable, however, that such useful structures in the ciliary method of feeding as eu-latero-frontal cilia once possessed would be lost, so long as the method of obtaining food remained the same, and then an attempt made to regain them, as exemplified by the anomalous latero-frontal cilia of the Ostreidae. This is the more unlikely as the mainly deposit feeding Protobranchs, N u c u l a and N u c u l a n a, have retained eu-latero-frontal cilia, though having no great use for them. The fact that in the second line (with micro-latero-frontal cilia) the most highly developed latero-frontal cilia—the anomalous—are found in the highest family of the group, the Ostreidae, points to the evolution of these structures within the group, and it would seem that the similarity both of structure and function between the anomalous and eu-latero-frontal cilia is due to convergence.

It may thus be conjectured that the remote ancestors of the 'sedentary' branch are to be sought in forms other than the Nuculidae, or any Protobranch family with living representatives, for these all have eu-, if not also pro-, latero-frontal cilia. Judging by the persistence of eu-latero-frontal cilia in the Nuculanidae and Nuculidae on gills which play only a subsidiary part in feeding, as well as by the uniformity of composition of the latero-frontal tract within existing families of Lamellibranchs, it seems improbable that extinct members of the Nuculidae, Nuculanidae, and Solenomyidae differed much from living members in the form of their latero-frontal cilia. But it

may be that some ancient, extinct families of Protobranchs were without eu-latero-frontal cilia, and gave rise not only to forms with such cilia, but also to those with micro-latero-frontal cilia. Douvillé (1912 *a*, pp. 443–4) noted that three families of Palaeoconchs, the Cardiolidae, with equivalve shells, the Antipleuridae and Vlastidae, with inequivalve shells, would be perhaps better placed in the 'sedentary' group, but he thought it probable that these are not to be considered as primitive forms (p. 421): it is therefore likely that they are not sufficiently generalized to have given rise to the 'sedentary' branch, and besides they are not known until the Silurian, while the Pterineidae occur in the Ordovician.

Thus, though the 'sedentary' branch of Lamellibranchs can be traced back to the Pterineidae (Ordovician to Carboniferous) yet their origin and relationship with other groups of Lamellibranchs remains obscure. It is an unfortunate truism that the taxonomic value of any character drawn from the soft parts of an animal is greatly restricted in that the position of fossils cannot be tested by such characters.

The Evolution of the Eulamellibranchiate or Synaptorhabdic Gill in the Group Characterized by the Possession of Micro-latero-frontal Cilia.

Within the group characterized by the possession of micro-latero-frontal cilia are two families, Pectinidae and Pteriidae, which, though characteristically filibranchiate or eleutherorhabdic, yet contain members with the synaptorhabdic tendency; one of these, the Pectinidae, and the forerunners of the other, the Pterineidae—and probably the Pteriidae themselves—have given rise to forms with eulamellibranchiate or synaptorhabdic gills (see Text-fig. 12, p. 414).

Most known members of the Pectinidae have gills with purely ciliary interfilamentar connexions, but the giant scallop P e c-t e n t e n u i c o s t a t u s Mighels has both organic and ciliary junctions, the latter occurring near the free margins—the youngest parts—of the gills (Drew, 1906, 1907 *a*), where, as will be seen later, in L i m a vestiges of ciliated discs are found.

From the Pectinidae in Carboniferous times arose the eulamellibranchiate family Limidae (Jackson, 1890, pp. 388, 391). The gills of Lima hians and Lima loscombi are curiously Pecten-like, the organic interfilamentar junctions having the appearance of spurs—such as are found in Pecten, Spondylus, and Amussium—which have fused. In Lima hians (fig. 1, Pl. 29) and Lima loscombi remains of ciliary junctions persist, being found mostly toward the ventral margins of the demibranchs, but the junctions are mainly organic. Ridewood (1903, p. 217) noted that in Lima inflata obsolete ciliated discs were merely suggested by the regularity of the prismatic epithelium.

An apparently unique use of interlocking cilia is found in the Limidae (Lima hians and Lima loscombi), in which the interfilamentar junctions do not run across the plicae as horizontal septa, as in Pinna and Ostrea, for instance. The interfilamentar junctions occur at regular and rather close intervals; they arise from the side of the principal filament and pass across the abfrontal surface of the ordinary and apical filaments to the next principal filament. The junctions are not directly transverse; they run in a series of V's, the apex of each being directed dorsally, and situated on an apical filament (Text-fig. 10). Long interlocking cilia, with the characteristic rotary movement of such cilia, are present on the intraplical face (i. e. facing the exhalent chamber) of that part of the interfilamentar connexion which extends across the filaments forming the crest of the plica (in Text-fig. 10 the apical and three adjacent filaments on each side). Under normal conditions the two arms of the V interlock (see fig. 1, Pl. 29), and hold the sides of the crests together, so that the plicae are steep-sided. Flattening of the plicae can only take place in that region devoid of interlocking cilia adjacent to the principal filaments, unless the cilia are torn apart, as shown in Text-fig. 10, which probably does not normally occur. Movement of the plicae is mostly a bending sideways of one fold toward the next, so that they partly overlap (see Text-fig. 11): only to a slight extent does widening and smoothing out of the plicae occur. It would seem that these ciliary junctions act in the manner of organic

Dorsal
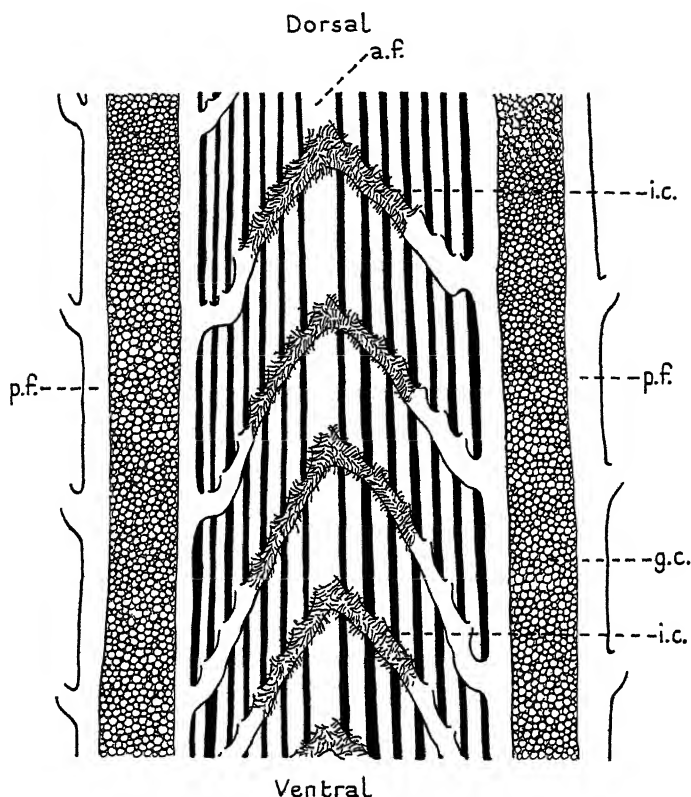
TEXT-FIG. 10.

Lima hians. Sketch from life of the abfrontal surface of a plica.
The plica has been opened out, so that the cilia normally inter-
locking on opposite limbs of the V-shaped interfilamentar junc-
tions, are shown in a non-interlocking position. (The number of
filaments to a plica in Lima hians varies between about 14 and
19.) *a.f.*, apical filament; *g.c.*, gland-cells of abfrontal surface of
principal filament; *i.c.*, interlocking cilia; *p.f.*, principal filament.
×93⅓.

intraplical septa—such as occur in Pinna and Ostrea—in
preserving the form of the plicae, and this method is a possible
precursor of organic union, a step in the tendency to progressive
firmness of the gill. The lacunar tissue of an organic horizontal

septum probably allows of greater play in increasing and decreasing the depth of the plicae than is possible by the ciliary method, and is without its weakness.

In the Pteriidae there are at least three species which show the synaptorhabdic tendency. In the genus P t e r i a , P t e r i a h i r u n d o has ciliary interfilamentar junctions (an instance of a principal and adjacent filament in organic union was observed, but this is unusual, and may have been due to proximity to the base of the gill), but in P t e r i a a r g e n t e a the junctions are of a compound nature, the intraplical edges of the spurs that bear the ciliated discs having fused (Ridewood, 1903, pp. 212–13). A similar condition has been described in P i n c t a d a v u l g a r i s (Herdman and Hornell, 1904, p. 60; Herdman, 1905, pp. 227–8), and has been found during the present work in P i n c t a d a m a r g a r i t i f e r a .

The Pinnidae, which arose in Devonian times, probably from L e p t o d e s m a , one of the Pterineidae (Jackson, 1890), has living members in which the gills have extensive organic interfilamentar junctions, they are in fact eulamellibranchiate, yet sections of P i n n a f r a g i l i s have shown that vestiges of ciliated discs still exist alongside the organic unions (fig. 2, Pl. 29), as in L i m a h i a n s and L i m a l o s c o m b i .

In P t e r i a h i r u n d o the interfilamentar junctions are entirely ciliary; in P t e r i a a r g e n t e a , P i n c t a d a v u l g a r i s , and P i n c t a d a m a r g a r i t i f e r a they are mainly ciliary with some little organic union; in P i n n a f r a g i l i s mainly organic with vestiges of ciliary junction. In the Pteriidae and Pinnidae there are therefore species the gills of which form a graded, though not a direct evolutionary, series.

A third eulamellibranchiate or synaptorhabdic family, the Ostreidae, has arisen within the group, but is somewhat apart from the rest—possessing anomalous together with para-laterofrontal cilia—though it obviously should be included on account of its derivation on independent grounds from either the Pteriidae or Pectinidae. Its gills are more highly evolved than those of either of the other two families with eulamellibranchiate gills, Limidae and Pinnidae, not only in the degree of development of the latero-frontal ciliated tract, but in the presence of organic
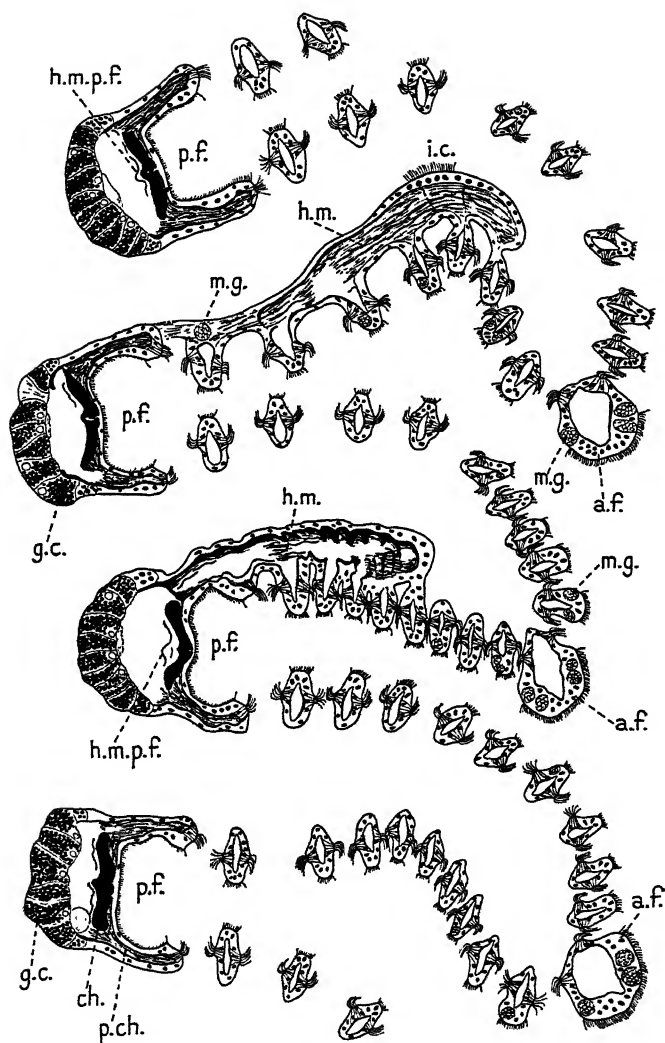
TEXT-FIG. 11.

Lima hians. Transverse section of three plicae and four principal
filaments of a lamella to show the bending sideways of the plicae.
a.f., apical filament; *ch.*, darkly staining chitin; *g.c.*, gland-cell

[For remaining description see opposite.]

fusion between the dorsal edges of the ascending lamellae of the outer demibranchs and the mantle, and between those of the inner demibranchs inter se; and in the absence of any vestiges of ciliary interfilamentar junctions, such as exist in Lima and Pinna (the first interfilamentar junctions of the gills of the spat of Ostrea virginica (Jackson, 1890, p. 304) and of Ostrea edulis (Yonge, 1926, p. 320) are organic). In the group under consideration the gills of Ostrea are the most definitely eulamellibranchiate in structure.

The immediate ancestors of the Ostreidae are doubtful; it is not known with certainty whether they have arisen from the Pteriidae or the Pectinidae. Jackson (1890, p. 307) thought it probable that Ostrea was derived from the Pteriidae, suggesting that it was descended either directly from Perna (=Isognomon) or a close common ancestor of the two genera, or from Avicula (=Pteria). Ostrea appeared in Carboniferous times, Isognomon is not known until the Trias (Dall in Zittel, 1913, pp. 450, 447), so that Ostrea is unlikely to have descended directly from Isognomon. The Pteriidae are present in the Silurian, Pteria itself in the Devonian; it is possible therefore for Ostrea to be descended either from Pteria, or some other member of the family. Dall (in Zittel, 1913, p. 455) followed Jackson in deriving Ostrea from the Pteriidae; Pelseneer (1911, p. 119) placed it as an offshoot from the Pteriacea: Davies (1933), on the other hand, considered that Ostrea shows by its hinge structure and muscle-plan its general affinity to Pecten. In 1853 Forbes and Hanley (vol. ii, p. 261) placed Lima, Pecten, Ostrea, and Anomia in the Ostreidae.

Gill structure unfortunately does not afford any help in determining the immediate ancestry of the Ostreidae. The gill axes are free for much of their length, but differ from those of

of abfrontal surface of principal filament; *h.m.*, horizontal muscle-fibres of the interfilamentar junctions; *h.m.p.f.*, horizontal muscle-fibres of the principal filaments; *i.c.*, interlocking cilia on intraplical faces of the interfilamentar junctions; *m.g.*, mucous gland; *p.ch.*, pale-staining chitin; *p.f.*, principal filament. Bouin-Duboscq's fixative; Mallory's triple stain. × 200.

the other two families with eulamellibranchiate gills, Limidae and Pinnidae, in the slight development of the longitudinal muscles. Vertical muscles in the demibranchs of Ostrea edulis are poorly developed: absence or paucity of such muscles is characteristic of the Pectinacea, including the Limidae, while the Pteriidae and Pinnidae usually have such muscles well developed. It is doubtful whether any relationship of the Ostreidae to the Pectinacea can be considered as indicated by the state of development of these muscles, for in Vulsella sp., belonging to the family Vulsellidae of the Pteriacea, vertical muscles are poorly developed, extending only a short distance from the gill axes into the principal filaments. The slight development of vertical fibres in Vulsella may possibly be correlated with the peculiarly sheltered life, embedded in sponges, and in Ostrea with a life of fixation from a very early age. Frontal currents similar to those of Ostrea are found on the gills of both Pecten and Pteria, in fact opposed frontal currents on all lamellae, frequently on the same filament, are common in the group with micro-latero-frontal cilia, the only known exception being Pinna (Atkins, 1936, 1937, 1937a).

In the group with micro-latero-frontal cilia, eulamellibranchiate gills, so far as is known, are always plicate and heterorhabdic, in contrast to those of the group with eu-latero-frontal cilia, which are frequently flat and homorhabdic. It seems that in the former group the growth of organic junctions has only occurred in gills which had already developed plications, while in the latter group it has occurred also in the flat-gilled stage. Flat homorhabdic and plicate heterorhabdic gills have been separated in Text-fig. 12, but though it seems possible, or probable, that the simpler condition generally precedes the more complex, yet the fact that the two conditions are found not only in the same family, but in the same genus (Pelseneer, 1911), suggests that the step from the one condition to the other is small. In the eulamellibranchiate genus Donax, which contains species with flat, with slightly plicate, and with strongly plicate lamellae, Rice, with what justification I do not know, regarded the simplicity of the flat forms as retrogressive (quoted

by Ridewood, 1903, p. 162): the possibility cannot be excluded that in filibranchiate gills the flat condition may in some instances be secondary.

To sum up, in the group with micro-latero-frontal cilia some families are still in the filibranchiate or eleutherorhabdic stage (Arcidae, Anomiidae, Pectinidae, Amussiidae, Spondylidae, Plicatulidae, Pteriidae, Vulsellidae, Isognomonidae), though showing an early stage in the transition to the eulamellibranchiate or synaptorhabdic condition in the compound junctions of Pteria argentea, Pinctada vulgaris, and Pinctada margaritifera, and considerable organic junction in Pecten tenuicostatus; others have attained the eulamellibranchiate stage (Limidae, Pinnidae, Ostreidae) but certain of them (Lima hians, Lima loscombi, Pinna fragilis) show in vestiges of ciliary junctions, signs of a passage through a filibranchiate stage (see Text-fig. 12).

In the group with micro-latero-frontal cilia there is clear evidence of the passage from the filibranchiate to the eulamellibranchiate condition. In the group with eu-latero-frontal cilia no such evidence has yet been demonstrated, though it is probable that existing Eulamellibranchs had ancestors with filibranchiate gills.

I think it is evident from the foregoing pages that gills at about the same stage of evolution occur in but distantly related groups of Lamellibranchs—that in the different lineages there is the same tendency towards consolidation of the gills—and therefore that the terms Filibranchia and Eulamellibranchia, Eleutherorhabda and Synaptorhabda, should only be used as descriptive of stages in gill evolution, and not logically as the names of orders. Gill structure is essentially a progressive character as already pointed out by Douvillé (1912a) and Davies (1933). Pelseneer (1911) held that the evolution of the gill may be considered as symbolizing the phylogenetic evolution of the Lamellibranchia: on this Douvillé (1912a, p. 423) commented: 'Les caractères tirés de cet organe sont bien certainement des caractères évolutifs, mais ... leur valeur phylogénique est très douteuse; les différents rameaux évoluent en effet d'une manière analogue, et ils doivent présenter la même succession
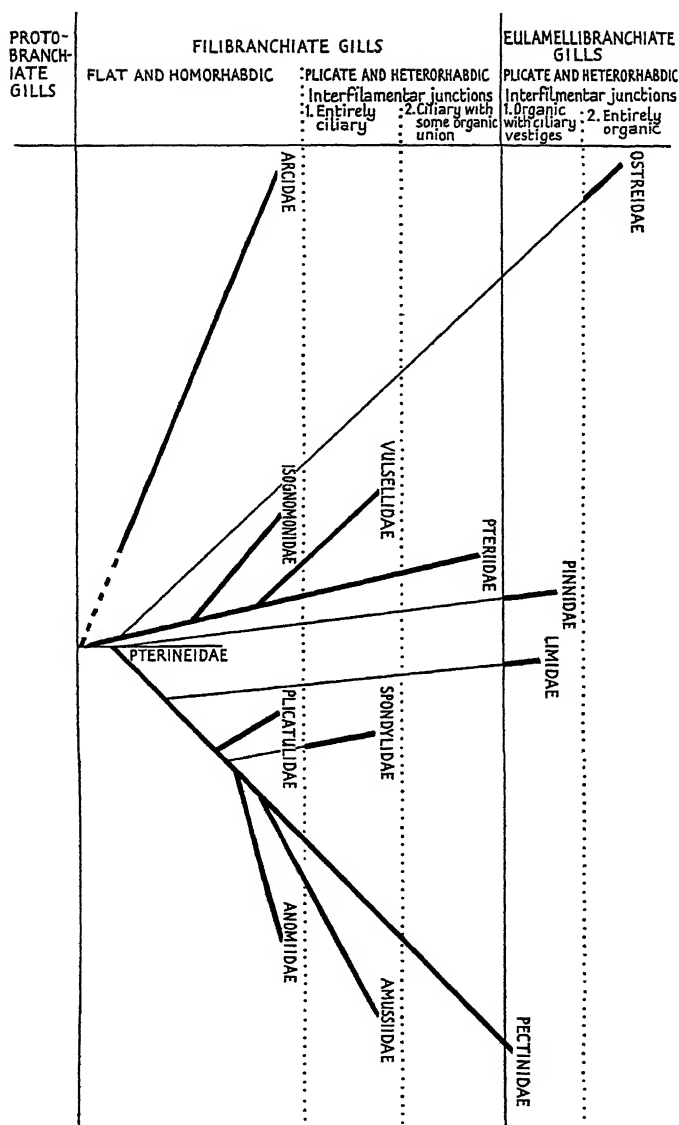
TEXT-FIG. 12.

Diagram showing the condition of gill evolution in the group of Lamellibranchs characterized by the possession of micro-laterofrontal cilia. When families have living members with the gills in a certain stage this is indicated by a thick line.

de caractères; ceux-ci ne peuvent donc permettre de reconstituer les rameaux.'

COMMON CHARACTERS OF LAMELLIBRANCHS POSSESSING MICRO-LATERO-FRONTAL CILIA.

Lamellibranchs within the group characterized by the possession of micro-latero-frontal cilia, have, in addition, a number of characters in common as given below. Not all these characters are restricted to members of the group, certain of them, which are probably primitive, occurring for example in those forms noted below which are apparently only distantly related. Three such characters are: (a) freedom of the posterior region of the gill axes (as also in aspidobranch Gastropods); (b) freedom of the dorsal edges of the ascending lamellae of the gills, or ciliary connexion only inter se and with adjacent parts; and (c) the absence of pallial sutures.

The Trigoniidae and Mytilidae, both of which were placed in the same order as the Arcidae and Anomiidae by Pelseneer, have some or all of these characters. In the Trigoniidae all three are found; in fact this family seems to be at about the same level of evolution as the Arcidae as regards gill and mantle freedom, though of different lineage. The Mytilidae, with certain exceptions (Mytilus ovalis, Musculus marmoratus, Septifer bilocularis (Ridewood, 1903)), agree with members of the group in the entirely ciliary method of division between the infra- and supra-branchial chambers, and in the relative freedom of the mantle margins, there being only a narrow pallial suture, but at least in Mytilus edulis and Modiolus modiolus, differ from them in the shortness of the free portion of the gill axis and its reattachment at the extremity.

Certain Eulamellibranchs exhibit some of these characters; in Astarte sulcata is found freedom of the posterior portion of the gill axis, and in the same region ciliary connexion of the gill inter se and with adjacent parts (Atkins, 1937a); there is also but one pallial suture. In the Solenidae and Solecurtidae (as represented by Solen marginatus, Ensis siliqua, Ensis arcuatus, Cultellus pellucidus, Solecurtus

scopula, and Solecurtus chamasolen), the gill axes
are free for a considerable distance, and this possibly occurs
in a number of Lamellibranchs, but masked by the fusion of
the dorsal edges of the ascending lamellae with some adjacent
parts. In the genus Venus (Venus verrucosa, Venus
casina) the gill axes are free for a short distance and then
reattached at the extreme tip as in Mytilus edulis.

As mentioned previously these are probably primitive lamelli-
branch characters, the retention of which has not been restricted
to any one group, though certain of them have tended to persist
to a greater degree in some groups than in others.

The common characters of the group with micro-latero-
frontal cilia are as follows:

1. 'Prodissoconchs of homogeneous laminar structure, but
not prismatic, and with umbos directed posteriorly'; the suc-
ceeding dissoconch with an external layer of prismatic cellular
tissue (Jackson, 1890). In this description Jackson did not
include the Arcidae, in different genera of which the umbos in
the adult may be directed forwards, inwards, upwards, or back-
wards (Reinhart, 1935). I have been unable to ascertain
whether the remainder of this definition is applicable to the
Arcidae.

2. Essentially byssiferous, leading to the greater development
of the posterior than the anterior region of the animal (Arcidae),
to reduction of the anterior region generally, and especially to
reduction (Pinnidae) and disappearance (many forms) of the
anterior adductor muscle (Douvillé, 1912 a). Byssal fixation is
not restricted to the group.

3. Considerable free posterior region to the gill axes. In some
forms (Malleus, Isognomon, Pteria, Pinna, Ostrea)
the gill axes are free for much of their length; in the Arcidae,
Anomiidae, and Pectinacea to a less extent. According to
Pelseneer (1911, p. 29) in the Pectens the extent of the free
posterior portion of the axis varies in different species, being
long in those with a stout byssus, and very short in free and
swimming forms. In the Ostreidae the dorsal edges of the
ascending lamellae of the outer demibranchs are fused with the
mantle, and those of the inner demibranchs inter se, so that

the condition of the gill axes is not seen without dissection. In Ostrea also the united dorsal edges of the two inner demibranchs are fused with the visceral mass anteriorly, for a distance varying between about a third and two-thirds of the total length in different species (Ostrea edulis, Ostrea virginica, Ostrea angulata).

In certain members (Arcidae, Anomiidae, Pectinacea) the gill axis is usually borne on a more or less deep suspensory membrane; such a membrane is absent generally in the Pteriacea (Pteria, Malleus, Pinctada, Pinna, Isognomon) and in the Ostreidae.

4. Considerable development of muscles in the gill axes, Ostreidae (Ostrea edulis) excepted. While characteristic of the group, this character is by no means restricted to the group.

5. Method of division of the supra- from the infra-branchial chamber: the division of the mantle chamber is either merely by the touching of the upturned edges of the demibranchs against adjacent parts, or by interlocking cilia, and rarely by organic junction.

It is advisable to discuss this character in some detail. Where the division is brought about merely by the touching of the dorsal edges of the ascending lamellae of the outer demibranchs against the mantle, and by those of the inner demibranchs inter se and against the foot or visceral mass, interlocking cilia are frequently present on the gills, though not on the parts they come in contact with: where the two inner demibranchs are in contact there is probably weak, easily dissolved, ciliary union. According to Bourne (1907) the short stiff cilia on the pallial faces of the velar filaments—that is the long reflected dorsal ends of the ascending filaments—of Anomia (Aenigma) aenigmatica function as ciliated discs, and give a sufficient amount of friction against the mantle—which is without corresponding cilia—to prevent the whole of the ascending lamella from slipping down. Division by mere touching of the gills against adjacent parts is found in the Arcidae (Arca, Glycymeris), Monia (Anomiidae), and Pectinacea. In Anomia ephippium and also in Anomia aenigmatica (Bourne, 1907) this method prevails between the outer demibranchs and

the mantle, but the inner demibranchs are in organic union. In a fragment of gills labelled Placuna placenta(?) from the British Museum, the union of the dorsal edges of the inner ascending lamellae was of a compound nature, being mostly organic, but with an exceedingly short ciliary junction to the ventral side of the organic fusion (fig. 3 A, Pl. 29). This type of junction has been described also in Placuna placenta by Hornell (1909, p. 70), while Ridewood (1903, p. 199) has stated for the same species that the two inner lamellae of the inner demibranchs are not united at their upper edges, the gills in this differing from those of Anomia ephippium, with which they otherwise agree exactly. It is possible that if material is alcohol preserved, the two edges might very easily be torn apart, or perhaps the method varies in different regions; one or other of these suggestions may possibly be the explanation of the conflicting statements.

Retention of the primitive condition of freedom both of the posterior region of the gill axes, and of the upturned edges of the demibranchs from adjacent parts, in the otherwise highly evolved Pectinacea has been possibly dependent on the active habits of many of them; it allows of the supra- and infra-branchial chambers being thrown into one during swimming, thus preventing possible injury to the gills. It is known that preparatory to the clapping of the valves in swimming the extremities of the gills swing forward by contraction of the longitudinal muscles of the gill axes, and this obviously necessitates freedom from connexion of the gills with adjacent parts.

Division of the mantle chamber by ciliary contacts is found in Heteranomia squamula (Anomiidae) (Atkins, 1936), and in the Pteriacea, though not in the Ostreidae. The contradictory statements of various authors as to whether the lamellae are free from, or attached to, adjacent parts in members of the Pteriacea may be explained by the ciliary nature of the junction allowing of fairly easy separation of the opposed surfaces. Grobben in 1900 (pp. 493–5) had already recognized the ciliary nature of the junction in Pteria and Pinctada, and considered it to be universal in the Pteriidae, in which he included Isognomon, Crenatula, and Vulsella, but

his paper seems to have been overlooked by Ridewood (1903) and Herdman and Hornell (1904, 1905). To the forms examined by Grobben may be added Malleus albus. According to Ridewood (p. 206) in this species 'the upper edges of the ascending lamellae are free from adjacent parts', but 'the apex of the ctenidium is fused with the mantle edge'. In a specimen received from the Indian Museum, Calcutta, the dorsal edges of the ascending lamellae of the two inner demibranchs were in ciliary connexion: those of the outer demibranchs were joined to the mantle by the same means, but the left outer demibranch had become partly detached, probably in opening the animal. In the region where the gill axes are free the interlocking was especially strong: sections through the gill and mantle failed to reveal any organic union. On the mantle margin, in the position of the posterior tip of the gill, fragments of lamella remained attached to the mantle after removal of the gill; sections, however, showed that the union was entirely ciliary, and that the tips of the gills are not fused with the mantle margin as Ridewood supposed, unless this occurs in some individuals and not in others.

In certain of the Pteriidae and in the allied family Isognomonidae there is undoubted slight organic fusion, as well as ciliary junction, between the two inner demibranchs. In Pinctada vulgaris while the junction is mostly ciliary, to the ventral side of this there is a narrow organic bridge (Herdman, 1905, p. 227): this is also found in Isognomon alata (fig. 3 B, Pl. 29). A junction of a compound nature has already been described in Placuna placenta (Anomiidae), but in that instance the relative importance of the two kinds of union was reversed (see fig. 3, Pl. 29). In Pteria macroptera a compound ciliary and organic junction is found between the two inner gills, but apparently only for a certain length behind the visceral mass (Pelseneer, 1911, p. 25).

In the Pinnidae the division between the two mantle chambers is generally by ciliary contacts. This was found to be so in unspecified Pinna by Grobben (1900, p. 495) and Stenta (1903, p. 228), in Atrina rigida by Grave (1911, pp. 418–19), and in Pinna fragilis by myself. In the latter species, at least,

there is a marked difference in the strength of the union between the gills themselves in the middle line, and between the gills and the mantle: the former junction is easily dissolved in the living animal, the latter most difficult to separate; indeed it was thought that the union was organic until sections were made. Ridewood (1903, p. 214) stated that in Pinna nobilis the upper edge of the outer ascending lamella is fused with the mantle, though in the four other species he examined (Pinna pectinata (=fragilis), Pinna nigra, Pinna zealandica, Pinna virgata), it was free; it is not impossible that he may have been misled by a strong ciliary junction such as occurs in Pinna fragilis.

The gills being long and the axes free for much of their length in Malleus, Pinna, and others, it is understandable that the junction of the gills with the mantle is necessarily strong. If such gills become separated from the mantle it would probably be difficult for them to regain their position. In Pinna the weakness of the ciliary junction in the middle line will allow the supra- and infra-branchial chambers to be thrown into one—thus preventing injury to the gills—during burrowing, when water is violently expelled from the shell anteriorly.

In Ostrea alone is the division between the two mantle chambers entirely organic. Ostrea, which is in all probability the most highly evolved member of the group, at least as regards the gills, for there are no vestiges of ciliary interfilamentar junctions, has moderate-sized or anomalous latero-frontal cilia: these differ in certain respects from the eu-latero-frontal cilia occurring in bivalves outside the group (see p. 365).

To summarize: division of the mantle chamber is (a) by touching of the upturned edges of the demibranchs against adjacent parts in the Arcidae and the Pectinacea, and in Monia among the Anomiidae. While this condition is found between the outer demibranchs and the mantle in Placuna and Anomia, between the two inner demibranchs there is a compound ciliary and organic junction in the former, and an entirely organic junction in the latter. (b) By interlocking cilia in Heteranomia squamula (Anomiidae) and the Pteriacea, though there is also some slight organic fusion between the

two inner demibranchs in certain Pteriidae and Isognomonidae. (c) By entirely organic junction in the Ostreidae.

Forms with the first method of division of the mantle chamber have each gill, or the united gills, free from adjacent parts: such gills are generally borne on more or less deep suspensory membranes. Forms with the second and third methods of division have the gills more or less firmly attached to adjacent parts: such gills generally lack suspensory membranes (see p. 417).

6. Gills with the outer and inner demibranchs similar, in that there is no supra-axial extension to the outer demibranch, though the ascending lamella of both demibranchs may be as deep as the descending. In the Ostreidae, however, the ascending may be rather deeper than the descending lamellae, and this is especially noticeable in the outer demibranch of O s t r e a a n g u l a t a.

In the unrelated family Mytilidae the outer demibranch is also without a supra-axial extension, and possibly also in the Trigoniidae.

7. In those species of which it has been found possible to obtain living material, namely, members of the Arcidae, Anomiidae, Pectinidae, Limidae, Pteriidae, Pinnidae, and Ostreidae, it has been found that longitudinal currents are present at the free ventral edge of both inner and outer demibranchs (except the outer demibranch of H e t e r a n o m i a), though these may be posterior in direction as in the Arcidae and Anomiidae. Also characteristic of the group, with the exception of the Pinnidae, is the presence of opposed frontal currents on a l l lamellae, frequently on the same filament (Atkins, 1936, 1937, 1937 a). Outside the group this arrangement of frontal currents is found in the Solenidae (Atkins, 1936). In the Mytilidae investigated longitudinal currents are present at the free ventral edges of both demibranchs, but frontal currents are entirely ventralward. The presence of opposed frontal currents on all filaments but of certain lamellae only in B a r n e a c a n d i d a, P e t r i c o l a p h o l a d i f o r m i s, S p i s u l a s u b t r u n c a t a, S p i s u l a e l- l i p t i c a, and C u l t e l l u s p e l l u c i d u s has been shown to be a special adaptation (Atkins, 1937, 1937 a).

8. Absence of pallial sutures. The mantle lobes are charac-

teristically free throughout their extent in the Arcidae, Ano-
miidae, Pteriidae, Isognomonidae, Amussiidae, Pectinidae,
Spondylidae, Plicatulidae[1] (see Watson, 1930, p. 25), and
Limidae. The right and left vela are fused for a certain distance
from the hinge ventrally in the posterior region in H e t e r a -
n o m i a s q u a m u l a (Atkins, 1936), and anteriorly in certain
species of L i m a without byssal fixation (e. g. L i m a h i a n s).
Slight fusion of the opposite mantle lobes in the region of the
posterior tips of the gills occurs in the Pinnidae and in the
Ostreidae. In L i m a h i a n s and L i m a l o s c o m b i there is
no fusion in this position, but the opposite vela are greatly
increased in depth locally to form projections, which approach
a pointed membraneous process of the postero-ventral region
of the visceral mass, and which, even when the valves are widely
gaping as usual in these species, make a partial, if not complete
division between the exhalent and inhalent currents.

The degree of fusion between the two mantle lobes is probably
more an adaptive character correlated with certain habits rather
than a progressive one. The retention of the primitive condition
of an open mantle in the group with micro-latero-frontal cilia
is probably dependent on their being typically surface forms.
Surface forms and mobile burrowers have retained a largely
open mantle in spite of one or two sutures, though many of these
among the Eulamellibranchs, have developed siphons. Such
siphons, however, rarely attain more than a moderate length,
and are generally partially or entirely united; when the siphons
are very long and separate this is an adaptation to deposit
feeding, as in the Tellinidae and Semelidae. An extensively
closed mantle, mostly though not invariably accompanied by
siphons, is found generally among burrowers and borers occupy-
ing more or less permanent holes, and perhaps has been developed
largely as a means of increasing the strength of the expelled
current on sudden closure of the valves, the fused mantle
margin being withdrawn into, and thus causing reduction of,
the infra-branchial chamber as described by Drew in S o l e n o -
m y a (1900) and E n s i s (1907). It may be that such forms

[1] No member of this family was examined, but there is no reason to
doubt that it is correctly placed in the Pectinacea.

need to be able to expel especially strong currents to keep their burrows clean and increase the depth: perhaps the arrangement compensates for the tendency of the adductor muscles toward contiguity to the hinge and to reduction in size, with consequent loss of power, evident in at least certain forms living in sheltered positions where there is no need for the shell to be tightly closed.

9. The inner fold of the mantle margin is commonly well developed, especially in swimming members where it forms a deep velum. The velum is deep in the Pectinidae, Amussiidae, Spondylidae, and Limidae; moderately deep in the Anomiidae, Pteriidae, and Ostreidae; narrow in the Isognomonidae; in Plicatula australis it is no more than 'a small ridge scarcely $\frac{1}{4}$ mm. in height' (Watson, 1930, p. 25).

10. The mantle is capable of withdrawing a considerable distance from the shell edge—that is, the retractor muscles of the mantle margin are inserted far from the shell edge—but to a much less extent in the Arcidae.

11. There is a tendency for members to lie on, or be attached by, the right valve.

Forms cemented by the right valve are, Spondylus, Chlamys distorta, and Plicatula australis.

Forms permanently attached by the byssus passing through a sinus in the right valve are, Anomia, Heteranomia, and Monia.

Forms temporarily attached by the byssus with the right side next the surface of attachment are, Isognomon, Malleus, Pteria, Pinctada, and certain Pectinidae.

Unattached forms lying on the right side are the free and swimming Pectinidae and the Amussiidae.

There is a difference of opinion as to the valve on which Placuna placenta (Anomiidae) lies: according to Hornell (1909, p. 46) it is the left convex valve; according to Fischer (1887, p. 933) it is the right. Jackson (1890) refers to Woodward as mentioning that, when young, Placuna has a byssal sinus in the right valve.

Apart from the uncertain case of Placuna, Ostrea seems to be alone in the group in lying on the left valve, which is cemented to the underlying surface. It is doubtful, however,

whether much importance should be attached to the habit of lying on, or being attached by, the right valve (for see Pelseneer, 1911, p. 86). O s t r e a is then exceptional in the composition of the latero-frontal ciliated tract; in the method of division of the mantle chamber; in the poor development of the longitudinal muscles of the gill axis; and in making attachment by the left valve.

Some free and some attached forms generally maintain the valves in a vertical position, for example, L i m a h i a n s, L i m a l o s c o m b i, and A r c a. When at rest beneath the surface G l y c y m e r i s appears to lie indifferently on the right or left valve, and has been found occasionally more or less vertical.

12. The presence of abdominal sense organs on the posterior adductor muscle (see p. 402). The position of these organs, however, appears to be correlated with the absence of siphons, abdominal sense organs occurring in this position in asiphonate forms outside the group with micro-latero-frontal cilia, for example, in the Trigoniidae (Pelseneer, 1906, p. 237) and in the Mytilidae (Field, 1922, p. 175).

13. The auricles intercommunicate; the Anomiidae excepted. Intercommunication of the auricles is also found in most Mytilidae (Pelseneer, 1911, pp. 95, 120).

Suggested Modifications of the Classification of the Lamellibranchia.

I am loath to add further names to the classification of the Lamellibranchia, while unable to divide phylogenetically the great group with eu-latero-frontal cilia, but I am inclined to the opinion that two groups, Macrociliobranchia and Microciliobranchia should be provisionally introduced. In the Macrociliobranchia are placed the Protobranchia, the Filibranchia (emended to include the Mytilacea and Trigoniacea only), the Eulamellibranchia (Pelseneer, 1911), and the Septibranchia. It is very probable, however, that the emended Filibranchia is still diphyletic, as already pointed out (p. 394). The Septibranchia may provisionally be classed as an order of the Macrocilio-

branchia: this seems warranted by their probable origin from the Anatinacea (with eu-latero-frontal cilia). The disappearance of latero-frontal cilia in this order is no doubt to be correlated with the change in the mode of feeding. Small latero-frontal cilia have been noted in Poromya oregonensis by Ridewood (1903, p. 274), but it is probable that these should be regarded as vestiges of eu-latero-frontal cilia, rather than as true micro-latero-frontal cilia.

As previously mentioned (p. 413), the terms Filibranch and Eulamellibranch indicate stages in gill evolution, likely to occur in different lineages, and therefore their permanent retention as names of orders, Filibranchia and Eulamellibranchia, does not seem desirable, but until the Macrociliobranchia can be divided according to genetic affinities they must be retained, though lamellibranchs with filibranchiate and eulamellibranchiate gills occur in the other group, Microciliobranchia.

In the Microciliobranchia the Anomiacea, Pteriacea, Pectinacea, and Ostreacea appear to be more closely related to one another than they do to the Arcacea, but whether this is sufficient to warrant the creation of two orders, the order Pseudolamellibranchia (emended) lapsing, I am unable to determine. In order to introduce as few new names as possible the order Pseudolamellibranchia has been retained in an emended sense, in spite of its unsuitability, leaving the introduction of a new name until such time as the revision of the Macrociliobranchia is undertaken. Pelseneer recognized the Pseudolamellibranchia as a natural group, in spite of filibranchiate and eulamellibranchiate members, but he missed the link that connects them, namely, the characteristic composition of the latero-frontal ciliated tract, and omitted to include the Arcidae and Anomiidae.

The suggested classification of the Lamellibranchia is then as follows:

Class LAMELLIBRANCHIA.

Group I. MACROCILIOBRANCHIA. Latero-frontal tracts of the gill filaments or leaflets consisting of a row of eu-latero-frontal cilia, with also a row of pro-latero-frontal cilia in all or most members.

Order 1. PROTOBRANCHIA (Pelseneer): Nuculidae, Nuculanidae, Solenomyidae.

Order 2. FILIBRANCHIA (emended). Restricted to Filibranchs with latero-frontal tracts of the type described for the group. This order is the order Filibranchia of Pelseneer, less the Anomiacea, the Arcidae, and allied families.

Sub-order 1. MYTILACEA (Pelseneer, 1911). This is the Mytilacea of Pelseneer, 1906, less the Pernidae.

Sub-order 2. TRIGONIACEA. Gill filaments with distinctive arrangement of the ciliary tracts, see p. 381. Trigoniidae.

Order 3. EULAMELLIBRANCHIA (Pelseneer, 1891, 1892, 1911, not 1906).

Order 4. SEPTIBRANCHIA (Pelseneer).

Group II. MICROCILIOBRANCHIA. Latero-frontal tracts of the gill filaments consisting characteristically of a row of micro-latero-frontal cilia.

Order 1. PSEUDOLAMELLIBRANCHIA (emended). This order is the order Pseudolamellibranchia of Pelseneer, 1911, emended to include those Filibranchs (Anomiacea, Arcidae, and allied families) with latero-frontal tracts of the type described for the group.

Sub-order 1. ARCACEA (emended). This sub-order is the Arcacea of Pelseneer less the Trigoniidae and allied families.

Sub-order 2. ANOMIACEA (Pelseneer).

Sub-order 3. PTERIACEA, including the Pinnidae (= Aviculacea of Pelseneer, 1911, less the Ostreidae).

Sub-order 4. PECTINACEA, including the Limidae (Pelseneer, 1911).

Sub-order 5. OSTREACEA. Latero-frontal tract consisting of anomalous together with para-latero-frontal cilia. Ostreidae.

Pelseneer (1911) and Douvillé (1912 a) have both given diagrams illustrating their conceptions of the phylogeny of the Lamellibranchia as a whole; Jackson (1890) gave only that of the Aviculidae and their allies.

In Pelseneer's diagram there is a single tree with many branches, and the divisions (orders) are horizontal and indicate stages in the evolution of the gills. The Filibranchia are shown

as derived from the Nuculidae; the Pseudolamellibranchia from the common ancestor of the Mytilidae and the Arcidae; the Eulamellibranchia from the Mytilidae by way of the Astartidae; and the Septibranchia from the Anatinacea. Comments on this classification have been made on pp. 394–398.

Douvillé, on the other hand, recognized three divergent branches from the beginning, corresponding to the three principal modes of life of the class, which he held early impressed on the branches certain characters which tended to persist through later secondary modifications. The three branches are:

(1) the 'normal' branch, more or less active and free living, descended from 'formes primitives nacrées normales' by way of forms like A c t i n o d o n t a;

(2) the 'sedentary' branch affected by byssal fixation, descended from 'formes primitives nacrées fixées' by way of the Pterineidae; the Arcidae being connected with these through P a l a e a r c a (=Cypricardites); and

(3) the 'burrowing' branch modified by a protected life in a more or less permanent burrow, descended from 'formes primitives nacrées cavicoles' by way of the Solenopsidae, Protomyidae, and Grammysiidae. His divisions are thus vertical. Davies (1933) has recently given a clear exposition, with diagrams, of Douvillé's scheme of classification and compared it with several other well-known classifications, so that there is no need to enter into it fully here. At present the only modifications of Douvillé's phylogeny that I am able to suggest are two: (a) the exclusion of the Mytilidae from the 'sedentary' branch. Though the condition of the latero-frontal tract shows that this family belongs to the Macrociliobranchia, yet it gives no indication of its allies, nor does the pattern of the lateral ciliated cells, which is not of the common type found among the 'normal' and 'burrowing' branches. Dall (in the Eastman edition of Zittel, 1913, p. 462) suggested that the prototypes of the Mytilidae are to be found in the Modiolopsidae: Douvillé placed M o d i o - l o p s i s in his 'normal' branch (though placing the Mytilidae in the 'sedentary' branch): this perhaps indicates where the allies of the Mytilidae should be sought. And (b) that possibly the 'burrowing' branch is not as widely separated from the

'normal' branch as is indicated in his diagram, a conclusion suggested by the character of the latero-frontal tract and the pattern of the lateral ciliated cells. A certain pattern of the lateral cells is common among both 'normal' and 'burrowing' branches, though it is not the only pattern found among these; the Mytilidae, Trigoniidae, Astartidae, Unionidae, and Aetheriidae having patterns different from the common one (Atkins, 1938a). I think the work recorded in this paper also shows that Nucula cannot be regarded as broadly ancestral to the 'sedentary' branch.

SUMMARY.

Certain Lamellibranchs have the latero-frontal tract composed of large complex 'cilia', here called eu-latero-frontal cilia, together with subsidiary ones, termed pro-latero-frontal cilia. This type of latero-frontal tract occurs in some or all of the three families of Protobranchs (there is some doubt as to the presence of pro-latero-frontal cilia in all the families), and in the Mytilidae and probably the Trigoniidae (fixation too imperfect for the identification of pro-latero-frontal cilia) among the Filibranchs, and in all the marine families of Eulamellibranchs obtainable at Plymouth, and in the fresh-water families, Dreissensiidae, Sphaeriidae, Unionidae, Mutelidae, and Aetheriidae. A list of the species investigated is given.

Other Lamellibranchs, which were previously considered as lacking latero-frontal cilia, have been found to possess small ones only, difficult of observation, termed micro-latero-frontal cilia. These occur in the Arcidae, Anomiidae, Pteriidae, Pectinidae, Spondylidae, Limidae, Pinnidae, and are inferred to be present in the Amussiidae, Vulsellidae, and Isognomonidae, in which eu-latero-frontal cilia are certainly absent. A list of the species examined is given.

In one family, the Ostreidae, moderate-sized latero-frontal cilia, termed anomalous latero-frontal cilia, together with subsidiary ones, termed para-latero-frontal cilia are present.

In bivalves having eu-latero-frontal cilia the arrangement of

the various ciliary tracts, frontal, latero-frontal, and lateral is fairly constant, notable exceptions being a Protobranch, N u c u l a n a , and a Filibranch, T r i g o n i a . In bivalves having micro-latero-frontal cilia the arrangement of the various tracts seems more or less constant.

The homology of the various types of latero-frontal cilia is discussed. The composition of the latero-frontal ciliated tracts has been found to be a stable character, and, as it is correlated with other characters, has taxonomic value.

It is suggested that the variations in the constitution of the latero-frontal tracts tend to show that Ridewood's (1903) classification does not express genetic affinities, as he himself conceded, nor does Pelseneer's (1911) entirely, and that Pelseneer's order Filibranchia, and Ridewood's orders Eleutherorhabda and Synaptorhabda are not monophyletic.

Families possessing micro-latero-frontal cilia appear to be closely related, and form a group, which, with certain modifications, corresponds to 'the Aviculidae and their allies', or the 'sedentary' branch of Lamellibranchs, previously established by the palaeontologists, Jackson and Douvillé respectively, largely on shell characters. Thus the constitution of the latero-frontal tracts of the gill filaments supports the findings of palaeontologists with regard to this group. Unfortunately neither Jackson nor Douvillé proposed a formal name for the group.

The relationship of forms with micro-latero-frontal cilia, and the evolution within the group of the eulamellibranchiate or synaptorhabdic gill are discussed. One family, the Ostreidae, which must be included on account of its relationship with either the Pteriacea or Pectinacea (based on other evidence) has moderate-sized, or anomalous latero-frontal cilia together with para-latero-frontal cilia. The anomalous latero-frontal cilia differ in certain respects from the large cilia characteristic of the majority of the Lamellibranchia, and are presumed to have arisen independently.

Common characters of the group characterized by the possession of micro-latero-frontal cilia, in addition to the form of the latero-frontal cilia, are: (1) shell characters of the prodissoconch,

Arcidae excepted; (2) byssal fixation; (3) considerable free posterior region to the gill axes; (4) considerable development of muscles in the gill axes, Ostreidae excepted; (5) method of division of the pallial cavity, Ostreidae excepted; (6) gills without a supra-axial extension to the outer demibranch; (7) presence of longitudinal currents at the free ventral edge of both inner and outer demibranchs; and of opposed frontal currents on all lamellae and frequently on the same filament, Pinnidae excepted; (8) absence of pallial sutures, Pinnidae and Ostreidae excepted; (9) inner fold of the mantle margin characteristically well developed, especially in swimming forms; (10) insertion of the retractor muscles of the mantle margin at a considerable distance from the shell edge, Arcidae excepted; (11) tendency for members, except the Ostreidae, to lie on the right valve; (12) abdominal sense organs on the posterior adductor muscle; and (13) intercommunication of the auricles, Anomiidae excepted.

Two groups of the Lamellibranchia are proposed provisionally, namely Group I, Macrociliobranchia, including the orders Protobranchia (Pelseneer), Filibranchia (emended to include only the Mytilacea and Trigoniacea), Eulamellibranchia (Pelseneer, 1911), and Septibranchia (Pelseneer); and Group II, Microciliobranchia, with the order Pseudolamellibranchia, emended to include the sub-orders Arcacea (excluding the Trigoniidae), Anomiacea, Pteriacea, Pectinacea, and Ostreacea. The Macrociliobranchia will need revision, for it is very probable that the Filibranchia (emended), if not the Eulamellibranchia, are still not monophyletic.

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EXPLANATION OF PLATE 29

Fig. 1.—L i m a   h i a n s. Transverse section of a plica to show vestiges of ciliary interfilamentar junctions (*i.c.*), ciliary junction between two limbs of a plica (*c.j.*), and muscle fibres. *a.f.*, apical filament; *ch.*, darkly staining chitin; *h.m.*, horizontal muscle-fibres of interfilamentar junctions;

h.m.p.f., horizontal muscle-fibres of principal filaments; *i.l.s.*, interlamellar septum; *m.g.*, mucous gland; *p.ch.*, pale-staining chitin; *p.f.*, principal filament; *v.n.*, vertical nerve. Bouin-Duboscq's fixative; iron haematoxylin and acid fuchsin. ×344.

Fig. 2.—P i n n a f r a g i l i s. Transverse section of three filaments, showing micro-latero-frontal cilia (*mi-l-f.c.*) and vestiges of ciliary inter-filamentar junctions (*i.c.*). *ch.*, chitin; *f.c.*, frontal cilia; *h.m.*, horizontal muscle-fibres; *l.c.*, lateral cilia; *p.ch.*, pale-staining chitin. Bouin-Duboscq's fixative; iron haematoxylin and acid fuchsin. ×ca. 262.

Fig. 3.—Transverse section of junction between dorsal edges of ascending lamellae of two inner demibranchs. A, P l a c u n a p l a c e n t a (?). The union is mostly organic but with a short ciliary junction ventrally. B, I s o g n o m o n a l a t a. The junction is mostly ciliary but with a short organic union ventrally. *c.j.*, ciliary junction; *ch.s.*, chitinous supporting structure; *i.c.*, interlocking cilia; *l.m.*, longitudinal muscle; *n.*, nerve; *t.m.*, transverse muscle-fibres. Alcohol fixation; iron haematoxylin and acid fuchsin. ×ca. 56.
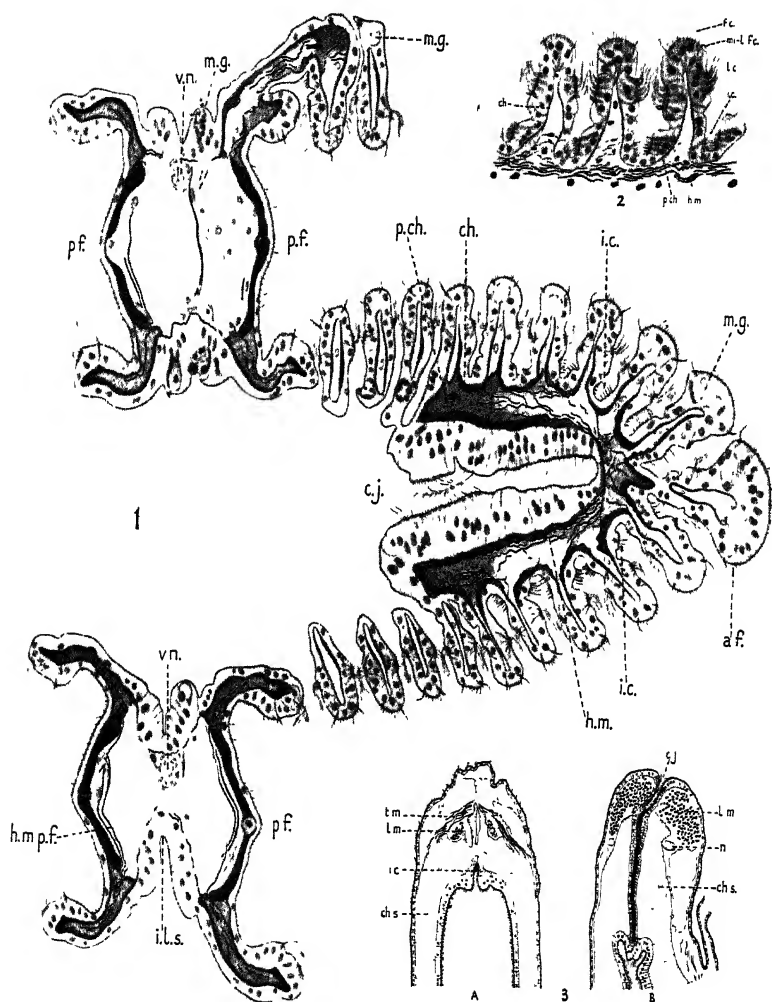
The Formation and Structure of a special Water-absorbing Area in the Membranes covering the Grasshopper Egg.

By

Eleanor H. Slifer,

Department of Zoology, State University of Iowa.

With Plate 30 and 5 Text-figures.

Contents.

Needham (p. 1272, 1931) divides all eggs into three classes: (a) those which, when first laid, contain too little inorganic matter and water for their complete development and which, consequently, must take in these substances, as well as oxygen, from the medium surrounding them; (b) those which are dependent upon their environment for additional water and oxygen; and (c) those which need only oxygen. Many insect eggs, available data indicate, belong to the second group, and during their development require, besides oxygen, a supply of water greater than that with which they are furnished at the time when they are laid.

As indirect evidence for such a view we may take those cases reviewed by Needham (pp. 904–5, 1931), Uvarov (pp. 70–3, 1931), and Buxton (pp. 306–13, 1932) where, with few exceptions, high humidities have been found to favour the develop-

ment of the eggs of various species of insects. Indirect evidence of another sort consists of observations on the increase in size of the eggs as they develop. It is probable that Réaumur (pp. 127–9, 1740) was the first to record this phenomenon. He described the eggs of a saw-fly as doubling in volume during their development, and wondered whether the shell of the egg might act as a 'placenta' to draw nourishment from the willow leaf to which it is fastened. He allowed leaves with eggs attached to dry, and states that the eggs soon shrivelled. The stems of other leaves with eggs on them were placed in water, and the eggs on these remained turgid and hatched from four to seven days later. Réaumur (1926)[1] noticed, also, that the eggs of ants increase in size as they develop. In the two centuries following Réaumur other workers have recorded such a size increase in the eggs of insects belonging to many different orders. Blunck (1914), Needham (1931), Johnson (1934), and Roonwal (1936) list numerous observations of this type.

In 1929[2]—and now the evidence becomes direct—Bodine published curves demonstrating that the increase in weight of the egg of the grasshopper, Melanoplus differentialis, as it develops, is due to an actual increase in water content. This was the first detailed, quantitative, and extensive study of the subject. Somewhat earlier Hoffman, Dampf, and Varela (1925) had reported a few experiments in which they showed that the eggs of another grasshopper, Schistocerca paranensis, which had been shrunken by exposure to dry air were able to absorb water and to regain their original turgidity when returned to a moist atmosphere. In 1930 Kerenski proved that the eggs of a beetle, Anisoplia austriaca, are able to increase in weight when moistened with nothing but water. Johnson (1934, 1937) has shown that the increase in size of

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[1] Réamur's notes on ants, overlooked for nearly two centuries, were finally found and prepared for publication by the late W. M. Wheeler.

[2] This article, as it appeared in *Physiological Zoology*, contained graphs from which the numbers had been removed through an oversight on the part of the publishers. The reprints, however, contain the numbers. This difference has been the cause of more than a little confusion in the literature.

the eggs of Notostira erratica is due to an absorption of water. Quite recently Roonwal (1936) has found, as had Bodine, that the water content of the grasshopper egg rises as it develops. Roonwal worked with the eggs of Locusta migratoria migratorioides.

Jahn (1935 a) has shown that the chitinous cuticle of the egg of Melanoplus differentialis is impermeable to such materials as $K_4Fe(CN)_6$ and $FeCl_3$. This cuticle, a secretion product of the serosa, is laid down during the fifth and sixth days of development at 25° C. and remains intact until shortly before the animal hatches (Slifer, 1937). The delicate, yellow, outer portion of the cuticle is highly resistant to wetting, and closely resembles the epicuticular portion of the body-wall of an adult insect; while the tough, white, inner portion contains chitin and has properties similar to those of the exocuticle and endocuticle of the adult's exoskeleton. In fact there is reason to believe that the chitinous cuticle represents the first embryonic exoskeleton. During the latter half of the incubation period, and after the embryo has undergone blastokinesis, a second embryonic exoskeleton is secreted. This is shed immediately after the insect leaves the egg. Since the outer yellow layer of the first embryonic cuticle is so resistant to wetting, and since it is present during all but the first few days of embryonic life, how, then, is water able to enter the grasshopper egg? The present paper deals with this problem.

PART I. THE FORMATION AND STRUCTURE OF A SPECIALIZED AREA IN THE CHITINOUS CUTICLE.

A number of years ago while studying the fatty acid content of the eggs of Melanoplus differentialis (Thomas) the present author noticed that the portion of the chitinous cuticle remaining after the eggs had been boiled in a strong solution of KOH showed at the posterior end a small, circular area which was so excessively thin as to be almost transparent. In contrast to this, the cuticle covering all other parts of the egg was, after such treatment, still tough, rather heavy, opaque, and of a whitish colour. The idea immediately presented itself

that this circular area might be specialized for the exchange of materials between the egg and its environment. Attempts to prove this hypothesis were made by coating the posterior end of the egg with such materials as paraffin and asphalt varnish. These experiments were unsuccessful—for reasons not apparent at the time but now quite clear—and the work was eventually abandoned.

After Jahn (1935 a, 1935 b) had published his observations on the properties of the membranes which surround the grass-hopper egg the presence of this thin area assumed a greater interest. It now seemed even more probable than before that this region might serve for the exchange of gases and liquids between the egg and its environment.

For microscopical studies a large number of Melanoplus differentialis eggs of various known ages were fixed in Bouin's or in Carnoy-Lebrun's solution. The eggs were sectioned longitudinally, at 7·5 microns, with the aid of the phenol water method described in an earlier paper (Slifer and King, 1933). Heidenhain's iron haematoxylin, Mallory's connective tissue method, and the Feulgen technique were used for staining.

A longitudinal section through the posterior tip of a 3-day old egg is shown in fig. 1, Pl. 30. It will be noticed that except at the extreme posterior end the chorion is thrown into depressions. These are the imprints of the ovariole epithelial cells which secreted the chorion. A fragment of one of the micropyles, which penetrate the chorion, is visible well back from the tip of the egg in the upper left corner of the drawing. The outermost layer of the chorion in the region posterior to the micropyles, is not clear and transparent, as it is elsewhere, but stains deeply and has a granular appearance. That this region differs from the rest of the surface of the egg can be demonstrated by placing whole eggs for a short time in Carnoy-Lebrun, then washing and staining in Mallory's connective tissue stain. The results are striking. The surface of the greater part of each egg, except in places where it has been injured, retains its original yellow colour, but the chorion covering the posterior tip is reddened with the fuchsin, indicating that the outer less permeable layer of the chorion is lacking here. In addition, the

contents of the micropyles stain brilliantly and, if the egg has been laid recently, each depression left by an ovariole epithelial cell will be filled with a blue-stained secretion, the so-called temporary coating of a previous paper (Slifer, 1937).

The cells of that part of the serosa which lies directly beneath the region of the chorion which contains no epithelial imprints are still thin and flattened in the 3-day-old egg (fig. 1, Pl. 30), but two days later they are found to be greatly enlarged, while the serosal cells elsewhere remain flattened. During the fifth and sixth days at 25° C. the serosa secretes the yellow cuticle over its outer surface (Slifer, 1937), and at the same time the enlarged serosal cells at the posterior tip of the egg secrete a membrane several times thicker than the rest of the yellow cuticle and distinctly different from it in structure (h, figs. 4, 5, and 6, Pl. 30). When examined with the aid of an oil-immersion lens this material, instead of appearing homogeneous, shows close-set and delicate striations running at right angles to the surface (fig. 4, Pl. 30). Following the formation of this striate layer the enlarged serosal cells begin the secretion of a second layer which is continuous with the white cuticle which covers the remainder of the egg and which is secreted by the ordinary serosal cells (Slifer, 1937). It never becomes as thick as the white cuticle found elsewhere but has the same structure, and stains in the same way.

Throughout the rest of this article this special area in the yellow layer of the chitinous cuticle will be referred to as the h y d r o p y l e (Gr. water-gate) and the enlarged serosal cells which produce it will be called the h y d r o p y l e c e l l s[1]. The introduction of these terms at this point is somewhat premature, but it will prevent much use of long descriptive phrases, and their suitability will become apparent towards the close of the paper.

By the time that the embryo is ready to undergo blasto-kinesis—which occurs on approximately the twenty-second day of active development at 25° C.—a considerable quantity of liquid has accumulated in the space between the white cuticle

[1] Roonwal (1935) noticed these enlarged serosal cells but was unable to assign them a function.

and the typical, flattened serosal cells found near the posterior
end of the egg (fig. 6, Pl. 30). The hydropyle cells are still
firmly attached to that portion of the cuticle which they secreted.
From this time on the history of the hydropyle cells can be
followed conveniently by making observations on the living egg.
This is best accomplished by placing an egg, from which the
chorion has been removed, in a drop of water on a cover-glass
to which several blobs of paraffin have previously been attached.
A smaller cover-glass is then laid over the egg and the whole
is inverted over the well of a depression slide. With such a
preparation it is possible to study the entire egg with a low-
power objective and the half (or more) near the observer with
a 4 mm. objective. Nuclei and cytoplasmic granules, vacuoles,
and filaments stand out with surprising clearness in the hydro-
pyle cells at the posterior end of an egg mounted in this way.
If it is desired to make a series of observations extending over
a long period the egg, when not being studied, may be removed
to an incubator and returned to the slide for examination at
selected intervals.

In order to learn the fate of the hydropyle cells eighty-one
eggs were chosen which were at the stage shown in Text-fig. 1 A.
These were studied with the help of the method just described.
During the initial stages of blastokinesis the strand of serosal
cells by which the embryo and yolk are attached to the hydro-
pyle cells becomes more and more slender (Text-fig. 1 B). Waves
of contraction, meanwhile, sweep along the lateral body-walls
of the embryo from the posterior towards the anterior end and
eventually the attaching strand breaks. In seventy of the
eighty-one eggs examined the break occurred in the thinner part
of the strand, the condition shown in Text-fig. 1 c resulting.
Here the hydropyle cells remain firmly attached to the inside
of the tip of the egg. The embryos in such eggs completed their
development and hatched, each leaving the hydropyle cells
behind inside the discarded yellow cuticle. In the remaining
eleven eggs the hydropyle cells were pulled loose entirely from
the end of the egg, and for some time could be seen as a
conspicuous lump on the serosa (Text-fig. 1 D). Later they
disappeared and were, presumably, taken into the mid-gut along
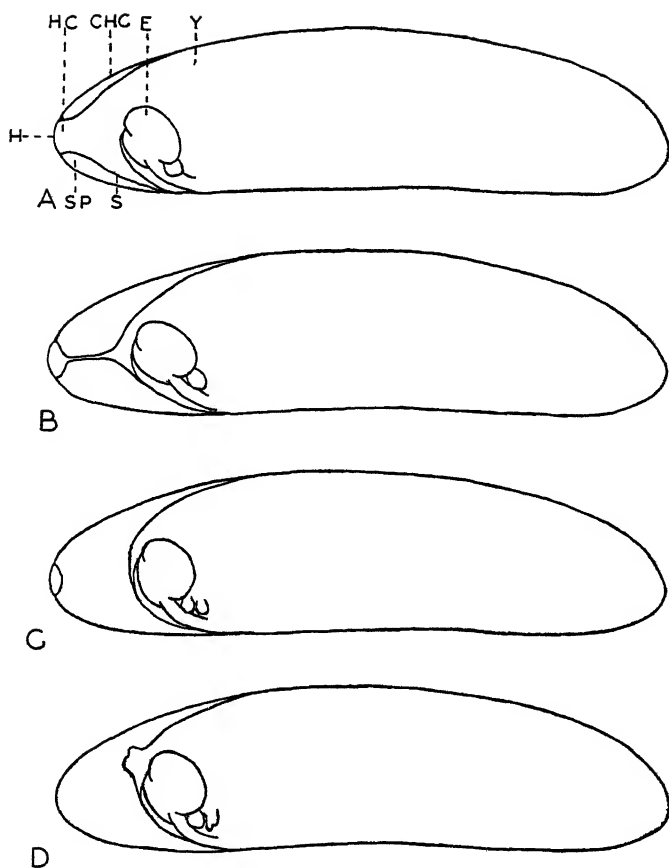
TEXT-FIG. 1.

Diagram representing Melanoplus differentialis eggs in stages preparatory to blastokinesis. The chorion has been removed from each egg. A, an egg in which the serosa (*s.*) has withdrawn from the chitinous cuticle (*chc.*) so as to leave a liquid-filled space (*sp.*). The head of the embryo (*e.*) is visible but the rest of it is covered by the yolk (*y.*). The hydropyle (*h.*) and the hydropyle cells (*hc.*) lie at the extreme posterior end of the egg. B, an egg at a somewhat later stage. C, an egg at a still later stage. The serosal strand has broken and the hydropyle cells remain attached to the inside of the posterior tip of the egg. D, an egg in which the hydropyle cells have been torn loose from the end of the egg and are visible, attached to the serosa, just above the embryo's head.

with the ordinary serosal cells and the yolk. Eggs in which this had occurred also hatched in an entirely normal manner.

From these results it may be concluded that the special function of the hydropyle cells is to secrete the hydropyle and that portion of the white cuticle which lies directly beneath it. After this has been accomplished they are no longer needed, and whether they remain attached to the posterior tip of the egg or are pulled away from it and digested makes little difference to the developing embryo. In eggs where the cells remain attached to the tip they may still be found, in sectioned material to retain a more or less normal appearance close to the time of hatching.

PART II. EXPERIMENTS ON THE REACTION OF THE HYDROPYLE AND OF OTHER PARTS OF THE EGG TO VARIOUS REAGENTS.

It has been stated in the preceding section that the thinness and unusual structure of the chitinous cuticle at the posterior tip of eggs which had been boiled in KOH, had aroused the suspicion that this particular area might be more permeable than other parts of the surface. With this in mind a number of reagents were selected which were apt to have some visible effect upon the surface or, should they succeed in entering, upon the contents of the egg. In all of the experiments which follow eggs were used from which the chorion had been removed. Observations were all made under a binocular dissecting microscope.

(A) KOH.

The effect of strong solutions of this reagent are especially conspicuous if eggs are used in which the embryos have just completed blastokinesis. The tip of the abdomen lies close to the posterior end of such an egg and a long, double row of cells containing white urate crystals is visible on either side of the abdomen. The alkali enters rapidly, and within a few minutes the extreme tip of the embryo's abdomen begins to show the first signs of disintegration. As the KOH diffuses inwards the urate crystals nearest the posterior tip of the egg suddenly disappear. When they have gone, those just anterior to them dissolve. This continues in a regular succession

from the posterior end forwards. The phenomenon is so striking and so orderly that only one explanation is possible. The alkali is entering through the posterior end and is diffusing anteriorly.

(B) Fixatives.

For experiments of this type eggs were used which were about to begin blastokinesis. If such eggs are placed in Carnoy-Lebrun the cells at the posterior end quickly coagulate and this coagulation—which is plainly visible—extends in a regular manner away from the posterior end. Eggs placed in Bouin's solution behave in the same way, but the fixative enters more slowly. Eggs exposed to 0·5 per cent. osmic acid are particularly interesting. The hydropyle turns brown almost instantly, then, very rapidly, becomes an intense black. Since the rest of the cuticle retains its original yellow colour the area blackened by the osmic acid stands out in sharp contrast.

(C) $KMnO_4$.

The yellow cuticle of an egg placed in a 0·05 per cent. solution of $KMnO_4$ soon turns brown while the hydropyle remains uncoloured. Eggs exposed to this solution for hours are unharmed and hatch at the usual time.

(D) $AgNO_3$.

Eggs about to undergo blastokinesis which are treated with 0·3 per cent. $AgNO_3$ and then placed in the sunlight retain their original colouring except at the hydropyle. Irregular brown patches appear slowly on this specialized area of the cuticle, and finally the whole circular area becomes black. The hydropyle cells also turned black in eggs which were exposed to $AgNO_3$ for an hour. After being washed these eggs were transferred to an incubator. No ill effects of the treatment could be observed. The eggs developed and hatched as usual. In some the blackened hydropyle cells were left attached to the posterior tip of the egg at blastokinesis and in others they were pulled away from it and, later, breaking loose from the serosa, were seen floating around in the fluid which bathes the embryo. The blackened cells from an egg of the former type were examined

in a yellow cuticle from which an embryo had just hatched. They were extremely hard and brittle.

(E) H Cl.

Eggs containing 17-day-old embryos were allowed to stand in concentrated HCl at room temperature for two weeks. At the close of this period the eggs had become coal-black but were still entire. The eggs were washed in distilled water and then transferred to 0·3 per cent. $AgNO_3$. At once a circular column of milky precipitate gushed from the posterior end of each. After a short time white spots appeared at other places on the surface of the eggs. These evidently marked the sites of small wounds. The forcibly ejected column appeared only at the posterior end. Eggs allowed to remain in the $AgNO_3$ solution eventually burst. This was clearly due to an inward passage of water more rapid than the outward diffusion of HCl. The hydropyle was examined microscopically in eggs which were removed to water from the acid and opened before bursting had occurred. No visible pores could be found in the hydropyle.

(F) H N O₃.

When placed in concentrated HNO_3 a white precipitate appears almost instantly at the posterior end of the egg and spreads rapidly away from it. A moment or two later the egg undergoes a miniature explosion, and a portion of the contents are thrown to a considerable distance through the ruptured hydropyle.

(G) Stains.

A number of eggs from which the chorion had been removed were exposed for a short time to Carnoy-Lebrun. Later these were washed and some treated with triosin and others with fast green. In both cases the hydropyle became brilliantly coloured while the rest of the cuticle was only faintly tinged by the stain.

Part III. The Effect of Applying an Impermeable Material to the Hydropyle.

From the experiments just described it is apparent that the hydropyle differs markedly from other parts of the cuticle in

its reaction to various non-biological materials. It remained to be seen whether such physiological materials as O_2, CO_2, and H_2O enter and leave with more ease at this special area than at other locations on the egg surface. The simplest way to solve such a problem would be to cover the hydropyle with some impermeable material, and to compare the subsequent history of such eggs with the history of other eggs similarly treated but with the impermeable material applied to some other spot on the egg. A number of impermeable or relatively impermeable materials were tried, but only one gave satisfactory results. This was a commercial product sold under the name of O.K. Liquid Solder. This solder dries very quickly, sticks to the cuticle with great tenacity, has no toxic effects, and proved to be very impermeable to water. In all of the experiments described below the chorion was first removed from the eggs. If this were not done the sponge-like chorion (which often has minute cracks in it by this time) would very readily conduct water up under the solder and so defeat the purpose of the experiment. Moreover, in eggs from which the chorion has been removed it is possible to observe the condition of the embryo as often as desired through the transparent chitinous cuticle. Such eggs are much more delicate than ordinary eggs, and extreme care must be taken in handling them. If the slightest wound is made in the cuticle a portion of the liquid bathing the embryo oozes out and hardens into a small red spot. Eggs of this sort, unless the injury has been too great, develop normally; but the presence of these spots would be a possible source of error in experiments of the type to be described below. Consequently all eggs which developed red spots were discarded at once.

(A) Respiration.

One series of 20-day-old and one series of 22-day-old Melanoplus differentialis eggs in which the diapause had been broken by exposure to low temperature were prepared as described above. The tips of the posterior ends of some, and the tips of the anterior ends of others, were then dipped in solder. The amount of oxygen consumed by each lot was then

determined in Warburg respirometers.[1] The results are shown in Table I. The figures are remarkably close and leave no doubt that the yellow cuticle as a whole is readily permeable to both O_2 and CO_2.[2]

TABLE I.

Two series of experiments in which the rates of oxygen consumption of post-diapause Melanoplus differentialis eggs with the anterior ends covered with solder were compared with the rates of oxygen consumption of similar eggs, the posterior ends of which were covered.

Stage of develop-ment of embryo.	No. of eggs.	Anterior ends covered.	No. of eggs.	Posterior ends covered.
20-day	59	0·21 mm.$^3O_2$/egg/hr.	136	0·21 mm.^{3}/egg/hr.
22-day	43	0·41 „ „	55	0·39 „ „

(B) Weight (Water-content).

Bodine's (1929) curves demonstrating the increase in the water-content of the eggs of Melanoplus differentialis as they develop show a gradual but steady rise from the time when the eggs are laid to the onset of diapause at the close of the third week of development at 25° C. The curves then flatten out and remain so for a long period. At the end of diapause the water-content again begins to rise, and does so with in-creasing velocity until the eggs hatch. The curves secured by the present writer for eggs of the same species agree in their general trend with those of Bodine, but—owing perhaps to the closer intervals at which determinations were made—two rather interesting differences have been found. As may be seen in Text-fig. 2 (which is based on data secured when the eggs were

[1] The author is indebted to Dr. E. J. Boell who very kindly made these determinations.

[2] It might be objected that the solder had not been shown to be im-permeable to gases. To test this point a piece of glass tubing tapering to a capillary at either end was filled with carbon dioxide gas. One capillary end was sealed in a flame and the other covered with the liquid solder. The piece of tubing was then suspended in a corked test tube over a solution of brom-thymol blue. At the end of seventeen hours the colour of the indicator still matched that of the standard. The tubing was then removed, a fine needle prick was made in the solder, and the whole quickly returned to the test tube. Within a few moments the indicator changed from bluish-green to a distinct yellow.

analysed for fatty acids, but which have not been published previously) the average wet weight of the eggs remains almost constant during the first week at 25° C. instead of increasing rapidly from the first day as Bodine's curves would indicate. This is a point of considerable interest, for it is at the close of the
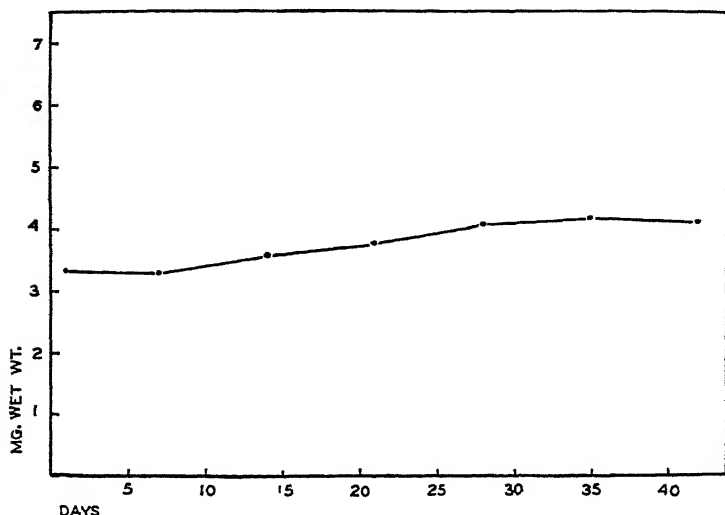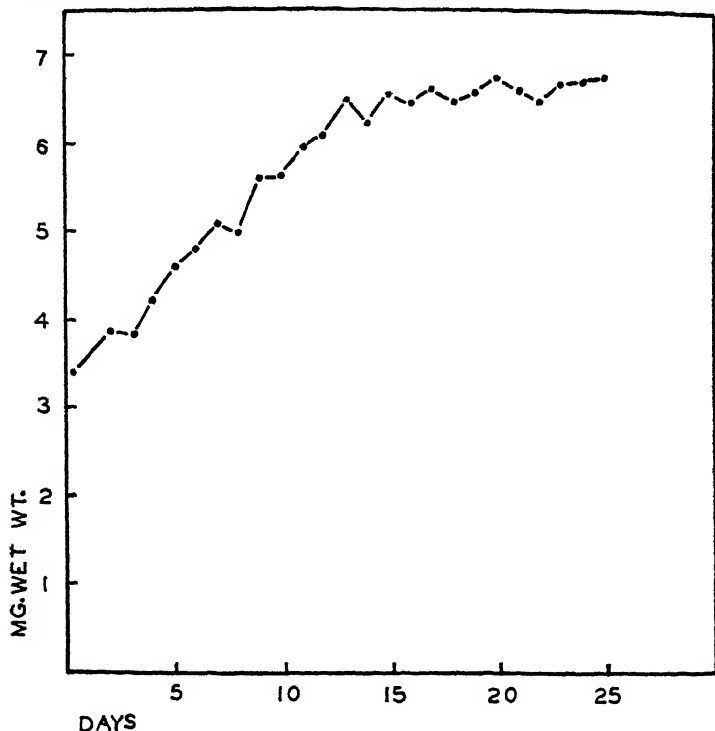

TEXT-FIG. 2.

Curve showing the increase in wet weight of the eggs of Melano-plus differentialis kept at 25° C. from the day when they were laid. The eggs enter diapause during the fourth week. Each point on the curve represents the weighted average for twenty experiments. A total of 20,441 eggs was used in securing the data for this curve.

first week that the serosa completes the secretion of the yellow cuticle. The acquisition of the power to absorb water in significant quantities is almost exactly coincident with the formation of this membrane.

In one other respect the curves secured by the present author differ from those given earlier. Instead of rising with increasing rapidity between the time when diapause is broken and hatching, as do Bodine's, those of the present writer show a rapid rise just before, during, and after blastokinesis. This is succeeded by a period of slower water uptake during the later stages of

incubation. The curve, some days before hatching, shows a
tendency to flatten out parallel to the base line. It is charac-
teristically concave rather than convex to the abscissa during

TEXT-FIG. 3.

Curve showing the increase in wet weight of Melanoplus dif-
ferentialis eggs during the last twenty-five days of incubation
at 25° C. These eggs had been exposed previously to low tempera-
tures in order to prevent the occurrence of a diapause. Hatching
began on the twenty-fifth day (thirty-eighth day of active
development at 25° C.). Each point represents a weighted average.
A total of 14,086 eggs was used in securing the data for this curve.

this period (Text-fig. 3). As the egg increases in size due to the
uptake of water the pressure inside the egg naturally increases.
The membranes are capable of considerable stretching, but after
a time the resistance to further stretching tends to balance the
forces leading to the further imbibition of water and the two

approach an equilibrium. The great turgidity possessed by the egg during its later development is well known to any one who has attempted to operate on eggs of this sort. A good portion of the egg contents spurt out at a slight prick of a needle.

For the experiments designed to test the effect of an impermeable coating applied to the hydropyle, eggs were chosen which had been kept at 15° C. for seventy-one days after they were laid. Such eggs contain embryos at a stage comparable to that reached after seventeen days at 25° C. (Slifer, 1932). These, when placed at 25° C., begin to develop more rapidly, and the majority hatch about twenty days later. The occurrence of a diapause is thus avoided. Seventeen-day-old eggs are particularly suitable for this purpose for the water-content is still low and the white cuticle is so well developed that the chorion can be removed without much difficulty. In the first few experiments of this type the eggs were weighed daily, but the eggs are delicate and the handling and drying preliminary to weighing caused the appearance of many small scars on the cuticle. It was necessary to discard so many eggs because of this that a new set of experiments was begun in which the weighings were made at longer intervals. The results of this experiment are shown in Text-fig. 4. Curve A shows the increase in weight of normal, untreated control eggs; curve B shows the weight of eggs from the same lot, the anterior ends of which had been dipped in solder, and curve C shows the weight of eggs the posterior ends of which had been similarly coated. Only one conclusion is possible. An impermeable covering applied to the posterior end almost completely prevents the uptake of water, while a similar coating on the opposite end has no such effect. Curve C does show a slight rise. This may be attributed either to some slight permeability of the yellow cuticle, or of the solder, to water, or to minute, unnoticed injuries of the yellow cuticle. Water, it may be concluded, enters (or is lost) in significant quantities through the hydropyle and not elsewhere.

(C) Development.

In addition to the weight measurements made on the eggs used in the experiments described in the preceding section,
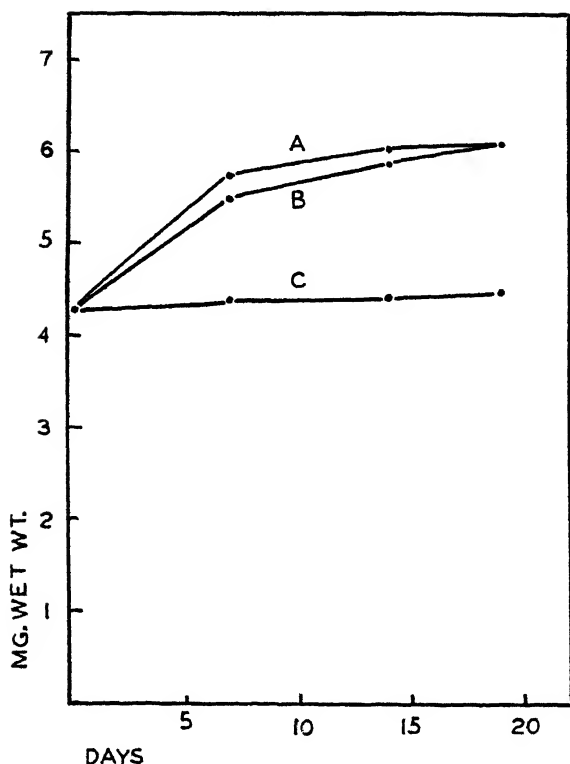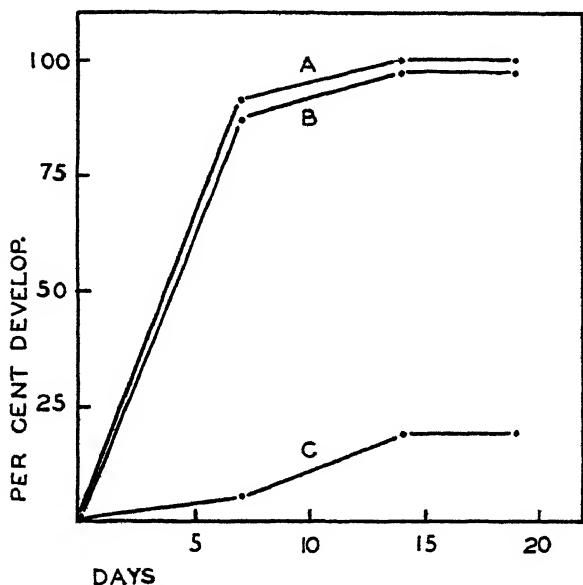
TEXT-FIG. 4.

Curves showing increase in wet weight of eggs with and without
the hydropyle covered with solder. A, untreated, control eggs;
B, eggs from which the chorion had been removed and the
anterior ends of which had been covered with solder; C, eggs from
which the chorion had been removed and the posterior ends of
which had been covered with solder. Curves B and C have been
corrected for the loss in weight due to the removal of the chorion.
The eggs at the beginning of the experiment were at the 17-day
stage of morphological development. Hatching, in the controls,
began on the nineteenth day of the experiment. 199 eggs were
used in securing the data for these curves.

observations were made on the course of development. Two
useful and conspicuous landmarks in the latter part of the
embryo's history are (1) the occurrence of blastokinesis and

(2) hatching. In Text-fig. 5 the percentages of the total number of eggs which had completed blastokinesis are given for the same eggs whose weights are shown in Text-fig. 4. The number of embryos which succeeded in completing blastokinesis is very much lower in the eggs which had the posterior ends covered

TEXT-FIG. 5.

Curves showing percentage of eggs in which blastokinesis had been completed. Eggs from same experiment as those in Text-fig. 4. A, normal, untreated controls; B, eggs with anterior ends covered with solder; C, eggs with posterior ends covered with solder.

than it is in either of the other two groups. By the twenty-first day, at 25° C., 73·5 per cent. of the control eggs, 60 per cent. of the eggs with the anterior end covered, but only 2·8 per cent. of the eggs with the posterior end covered had hatched. Development, then, stops at once or progresses at a greatly retarded rate in eggs when the entrance of water is prevented. It is conceivable, of course, that the solder placed in such close proximity to the head of the embryo might have some toxic effect. This objection was met by removing the solder from twenty-seven

of those eggs which had had the posterior end covered for four weeks, and which had showed no signs of developing during that period. This was easily accomplished by using acetone as a solvent for the solder. One week later 60 per cent. of these eggs had completed blastokinesis.

One point remained to be settled. Would the application of solder to the posterior ends of eggs after the greater part of the water had already been taken up have any retarding effect on development? This question was answered by placing solder on the posterior tips of thirty eggs which were scheduled to hatch about a week later. A like number had the anterior ends covered. The results were clear-cut. All hatched at the same time as the controls. The solder, then, does no harm. It is only the lack of water which stops or slows down development.

DISCUSSION.

A search through the literature on embryology in an attempt to learn whether or not the special area in the chitinous cuticle, here called the hydropyle, is present in other insect eggs has produced little in the way of results. It has, apparently, been the practice of nearly all those who have worked with insect eggs to remove the secreted membranes while preparing them for study. Structures such as the curious 'dorsal organ', of unknown function, described for certain apterygote insects, as well as for the eggs of a number of arthropods other than insects,[1] arouse the suspicion that this may correspond to the mass of cells which secretes the hydropyle in the eggs of Melanoplus differentialis. For example, the 'precephalic organ' described for the egg of Anurida maritima by Imms (1906) and the 'indusium' found in eggs of the Paratenodera sinensis by Hagan (1917) both bear a strong resemblance to the hydropyle cells of the grasshopper egg. But the illustrations accompanying the descriptions are not sufficiently detailed to tell whether or not such a special area exists. Moreover, little is known concerning the water metabolism of these forms.

More data, pertinent to the problem under discussion, are

[1] Hirschler (1928) and Korschelt and Heider (1936) may be consulted for references which deal with these structures.

available for the eggs of Notostira erratica than exist for those of most other forms. The eggs of this hemipteran absorb water, as Johnson (1934, 1937) has demonstrated, and a comparison of Johnson's figures of sectioned eggs (especially of fig. G, pl. II, 1934) with fig. 6, pl. 30 of the present paper is illuminating. The cells which Johnson describes as 'columnar epithelium of cells lining the yolk-plug wall' closely resemble the hydropyle cells of the grasshopper egg. The two-layered 'yolk-plug membrane' corresponds, apparently, to the chitinous cuticle of the present paper but it is not possible to tell, from Johnson's figure, whether the membrane is modified above the columnar epithelium to form a hydropyle. It would be interesting to test this point experimentally with the Notostira egg. Finally, Weber's (1931) description of the uptake of water by the eggs of a coccid, Trialeurodes vaporariorum, from the tobacco leaves to which they are attached by a small, thin-walled stalk should not be overlooked. A short time after the eggs are laid a brown membrane is formed below the chorion. This extends into the stalk and is thinner in that region than it is elsewhere. Weber states that when leaves with eggs attached to them are allowed to wither the eggs on them soon collapse and die—which recalls Réaumur's experiments, long ago, with the saw-fly eggs. After the inner brown membrane has formed the eggs are less sensitive to changes in the turgidity of the leaf than they are when newly laid. It seems probable that conditions in the coccid egg bear a rather close resemblance to those in the egg of the grasshopper.

Conclusions.

1. Water enters (or leaves) the egg of the grasshopper, Melanoplus differentialis, after the sixth day of development at 25° C., through a small, circular, specialized area in the yellow cuticle located at the posterior end of the egg. This area has been named the hydropyle.

2. The hydropyle is secreted by a group of enlarged and modified serosal cells. These are called the hydropyle cells.

3. The outer layer of the greater part of the chorion consists

of a clear material which is not readily permeable to dyes. At the posterior tip of the egg this impermeable layer is lacking.

4. The grasshopper egg first begins to absorb water in considerable quantities immediately after the yellow cuticle is formed.

5. Towards the close of embryonic development the rate of water uptake falls off markedly.

6. The chitinous cuticle as a whole is readily permeable to O_2 and CO_2.

7. If water is prevented from entering the egg by covering the hydropyle with a water-impermeable material development is stopped, retarded, or unaffected depending upon the water-content of the egg at the time when the impermeable coating is applied.

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EXPLANATION OF PLATE 30.

Fig. 1.—Longitudinal section through the posterior tip of a Melanoplus differentialis egg which has been kept at 25° C. for the three days since it was laid. The serosa (s.) and germ-band or embryo (e.) lie directly beneath the chorion (ch.) and at the surface of the yolk (y.). A fragment of a micropyle is visible in the chorion in the upper left-hand corner of the drawing. ×160.

Fig. 2.—Diagrammatic, lateral view of an egg, from which the chorion has been removed, to show the size of the hydropyle at the posterior end of the egg. The concave side of the egg represents the ventral surface. ×10.

Fig. 3.—Diagrammatic view of an egg, from which the chorion has been removed, as seen from the posterior end. ×10.

Fig. 4.—A section through the hydropyle at right angles to the surface of an egg fixed twelve days before hatching was due. Note the striated appearance of the modified yellow cuticle as seen under high magnification. ×1,678.

Fig. 5.—A section through a part of the hydropyle and a few of the hydropyle cells cut at right angles to the surface of an egg fixed twelve days before hatching was due. ×680.

Fig. 6.—Longitudinal section through the tip of an egg fixed shortly before blastokinesis (Text-fig. 1 A represents an egg at this same stage). The serosa (s.) has withdrawn from the white cuticle (wc.) leaving a liquid-filled space between the two. A few particles of yolk are enclosed within the serosa. m., fragment of micropyle; ch., chorion; yc., yellow cuticle; h., hydropyle; hc., hydropyle cells. ×160.
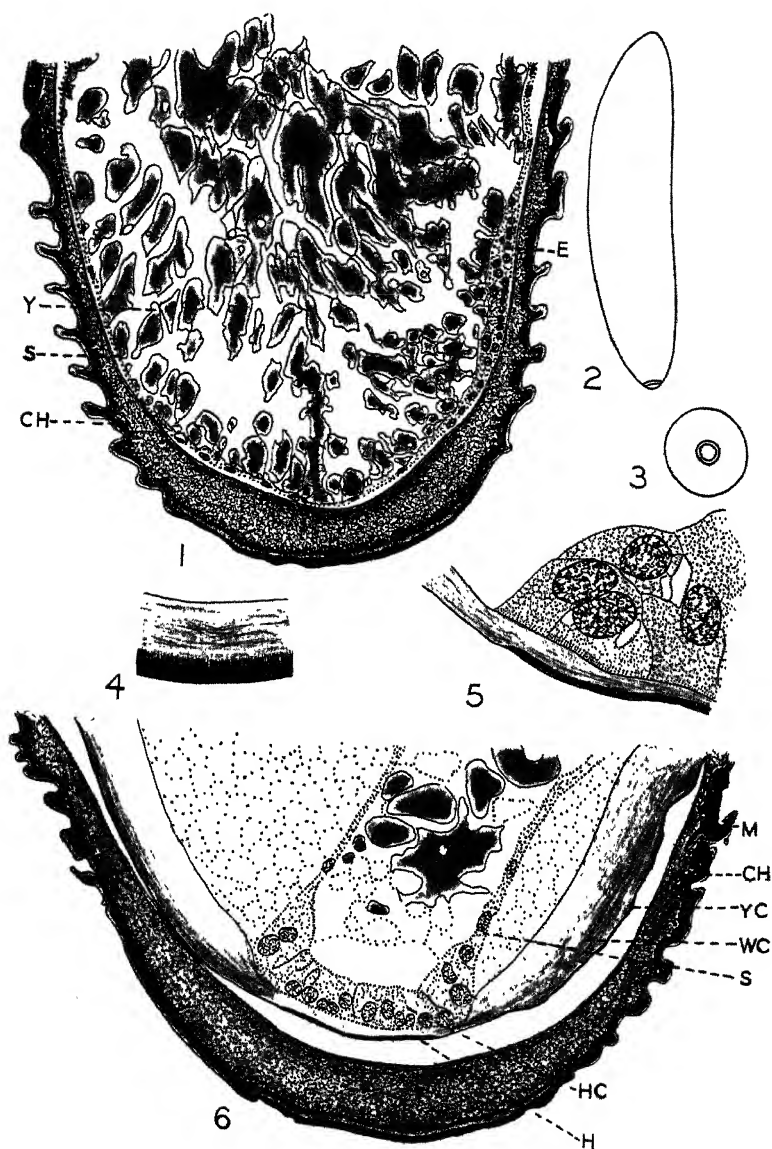
An Account of Amoeba discoides; its Culture and Life History.

By

Catherine Hayes, S.N.D., B.Sc.,

From the Zoology Department, University of Glasgow, and the
Laboratory of Notre Dame, Dowanhill, Glasgow.

With Plates 31 and 32 and 6 Text-figures.

INTRODUCTION.

SOME years ago Schaeffer (1916) pointed out that there was
great confusion concerning the description of Amoeba
proteus, the largest of the fresh-water amoebae. He then
proceeded to show that there were no less than three species of
amoebae indiscriminately referred to as Amoeba proteus.
There is no need to give here the details of Schaeffer's investiga-
tion and the reasons for his conclusions. It suffices to say that
he gave the names Amoeba proteus, Amoeba dubia,
and Amoeba discoides to the three large amoebae.

Dr. Lucy Carter, who, in 1910, at the suggestion of Professor
Graham Kerr, had undertaken to investigate the life-history of
Amoeba proteus, was confronted at the outset of her work
with that confusion of nomenclature to which reference has
already been made. She published the results of her experience
in a paper entitled 'Some Observations on Amoeba proteus'
(Carter, 1919). It seems quite clear from a scrutiny of the work
of these two authors that Carter's Amoeba proteus X
corresponds to Schaeffer's Amoeba dubia. Later Schaeffer
(1926) resuscitating the Linnaean genus Chaos gave the new name
Chaos diffluens to Amoeba proteus Pallas (Leidy)
(= Y, Carter). Amoeba dubia (= Amoeba proteus,
Penard = Amoeba proteus X, Carter) he named Poly-
chaos dubia, and Amoeba discoides he named
Metachaos discoides. In spite of Schaeffer's suggested
changes of nomenclature I shall use the old terms Amoeba

proteus, Amoeba dubia, Amoeba discoides in this paper.

Although I have long worked on Amoeba proteus (= Chaos diffluens) and collected material in the district around Glasgow, I have never come across Amoeba discoides. Indeed, so far as I have been able to ascertain, it has never been recorded in Great Britain and seems to be absent from the fauna.

SOURCE OF THE MATERIAL.

The material was kindly handed over to me by Mr. Watkinson and Mr. Sutcliffe, and to both these gentlemen I wish to express my very sincere thanks.[1]

I also take this opportunity of expressing my best thanks to Sister Monica for having given me the material, and for having placed at my disposal throughout the progress of the work her varied knowledge and experience in the cultivation of micro-organisms.

CULTURE OF THE MATERIAL CONTAINING AMOEBA DISCOIDES.

The amount of material at my disposal was limited. Its source was precarious, as any slight change in the conditions of the tropical fish tanks might kill off all the amoebae. Hence, it seemed advisable to establish good, strong, laboratory cultures before proceeding to fix large quantities preparatory to working out the cytology. The growth of all large free-living amoebae is slow, and it takes years to accumulate abundant material. Schaeffer (1916) said that Amoeba discoides

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[1] For many years Mr. Harry Watkinson of Grimsby and Sister Monica have exchanged material and notes on many problems connected with pond life and micro-aquaria. Three years ago Mr. Watkinson became interested in the organisms which he found in the fresh-water aquaria for rearing tropical fish, owned by the well-known aquarist Mr. Albert Sutcliffe of Grimsby, and endeavoured to make an ecological survey of each tank. While engaged on this work he came across a large free-living amoeba with which he was not acquainted. He had long possessed sub-cultures of Sister Monica's Amoeba proteus and was familiar with Amoeba dubia. He sent this unknown amoeba to Sister Monica for her inspection, who, suspecting that it was Amoeba discoides, asked Mr. Watkinson for more material which is that here described.

was a slower grower than Amoeba proteus. As subsequent readings from my field book observations will show, I cannot endorse this statement unreservedly.

The first stock of amoebae arrived in a 250 c.c. capacity bottle. This was allowed to stand over night so that the debris containing the amoebae had time to settle on the bottom. Then into a glass trough (diameter = 4 inches, height = $2\frac{1}{2}$ inches) was poured Glasgow tap-water to a height of about $\frac{1}{2}$ inch to which some of the supernatant fluid from the bottle containing the amoebae and eight wheat-grains boiled for five minutes to kill the embryo were added. To this were added amoebae from the surface of what remained of the original debris at the bottom of the bottle, great care being taken to avoid the silver sand which lay underneath and to take only the rich mud from its surface. The amoebae were added in groups at intervals of a day for several days.

The culture thus started on November 14, 1934 (called culture A), was successful and has now (July 1937) been sub-cultured. At intervals of three months five or six wheat-grains and a little water have been added to it.

The bottle in which the amoebae arrived with its remaining debris (chiefly silver sand) was kept and filled up with Glasgow tap-water. This was left undisturbed to be used for future sub-cultures, and also to replenish the liquid in culture A at intervals. Each time that water was taken from this bottle it was replaced by fresh tap-water so that there was always a supply of water which had stood for some time over what remained of the original debris. After the culture had been going for about a year, water straight from the tap was used to replenish it, for as Glasgow tap-water is so admirably adapted to Amoeba proteus culture it was deemed safe for this particular amoeba.

A second consignment of 350 c.c. of material arrived from Mr. Watkinson (November 22, 1934), and was found to contain many of the amoebae. The material was not removed from the bottle, but an attempt was made to cultivate the amoebae by adding occasionally very minute quantities of 'Spratt's Tropical Fish Food', on which Mr. Sutcliffe fed his tropical fish and of

which he had very kindly sent me a box. This experiment was a failure. The amoebae, plentiful at first, gradually disappeared, nor did they reappear as in the successful culture A. The entire disappearance of the amoebae may be due to the pabulum or to the fact of their having been kept in the bottle and not put out into flat vessels.

The amoebae evidently have died out in Mr. Sutcliffe's tanks as no further stock has been received.

During the time that I have been caring for and examining the cultures of Amoeba discoides I have inclined to the opinion that the most successful were those reared in Petri dishes. During the Session 1936–7 I have had cultures in glass troughs 6 inches diameter and 4 inches deep, and others in Petri dishes 4 inches diameter and ¾ inch deep. The culture water in the troughs was about 3 inches deep. Though these respective cultures were obtained by subdividing the same parent culture, and though they were submitted to the same technique the results are outstandingly different. The cultures in the troughs have gradually grown poorer until now (July 1937) they seem to contain no amoebae at all, while the Petri dish cultures are most luxuriant, containing large numbers of beautiful amoebae.

The pH of the water in which Amoeba discoides lives appears to have no great significance in the cultivation of this rhizopod, if we except the fact that it is always lower than pH 7. Several good cultures vary between pH 6·5 and pH 6·8, but I have also a luxuriant culture whose present pH (Aug. 1937) is 4·5.

I have not been able, however, to perform any micro-injections to test the pH of the cytoplasm.

### DESCRIPTION OF THE LIVING ADULT AMOEBA DISCOIDES (SCHAEFFER.)

As will be explained more fully later in this paper, the culture undergoes 'depression' periods and 'optimum' periods. A 'depression' period is one during which the culture contains no adult amoebae. Conversely during an 'optimum' period it contains large numbers of adult amoebae. Definition of these two terms

is necessary at this point so that it may be clearly understood what I mean by 'young' adult amoebae and 'old' adult amoebae. When the full grown amoebae first appear in the culture after a 'depression' period I call them young adults. These live for from three to six or even seven months, during which time they increase by fission. Now comes a time when they begin to disappear gradually from the culture and when they exhibit features not seen three months earlier. At this stage I call the amoebae 'old' adults.

If a pipette full of the culture containing young adult amoebae be transferred to a glass cell and examined at once over a black background under the low power of a Zeiss Greenough binocular an observer at first experiences difficulty in recognizing the amoebae. A little patience brings its reward for these fantastically shaped and exceedingly translucent creatures make a beautiful picture as they lie emmeshed in the green algae. They possess numerous pseudopodia stretching outwards in all directions. When these same amoebae are placed on a slide in a drop of culture water under a cover slip, given time to grip the substratum and are then examined under a microscope with transmitted light, the shape is found to have changed completely (fig. 1, Pl. 31). They are no longer radial but long and flat, the pseudopodia being fewer in number and in one plane (Text-fig. 1, a, b, c). The cytoplasm of the healthy amoeba spreads out over a large surface area and consequently has but little depth or thickness. This makes the examination of the living nucleus and the cytoplasmic contents easy. The average length I found to be 420μ (Schaeffer (1916) gives 400μ as the average length): when the amoeba is stretched out to 420μ its width is about 140μ. The cytoplasm is very finely granular, exceedingly mobile and flows with great rapidity. The flow or movement is always in the centre both in the main bulk of the creature and along the pseudopodia. No forward movement of the cytoplasm at the sides can be detected. When the amoebae are fully stretched out there are no folds in the cytoplasm. The ectoplasm is exceedingly sparse, forming only a thin skin round the endoplasm. Even at the tips of the pseudopodia ectoplasm is only rarely seen. It is most conspicuous at the base of the

pseudopodia, that is, in the angle between the main body and a pseudopodium or between two pseudopodia.

Embedded in the cytoplasm is a number of crystals and many minute particles which may be nascent crystals. These
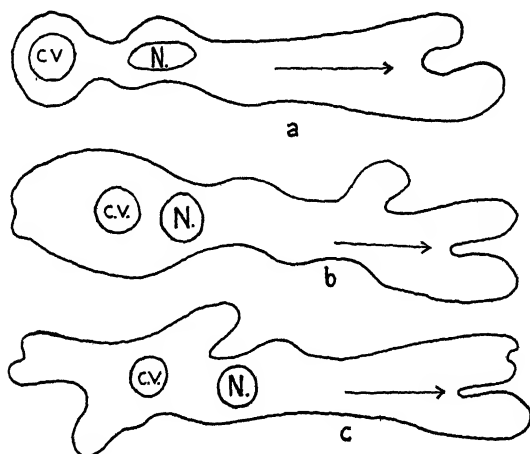

TEXT-FIG. 1 *a*, *b*, and *c*.

Three outline drawings (not to scale) to show the usual forms assumed by Amoeba discoides when it has been a little time on a slide under a cover glass. *c.v.*, contractile vacuole; *N.*, nucleus.

crystals resemble the characteristic bipyramidal ones found in Amoeba proteus but they are much less truncated than those of Amoeba proteus, also the angles or edges are less sharp and the whole crystal has a more oval appearance. I cannot agree with Schaeffer that this amoeba is more stuffed with crystals than Amoeba proteus. During the many years that I have worked in the Notre Dame Laboratory I have examined innumerable Amoeba proteus, and it is my experience that under certain conditions of culture and age the crystals are far larger and more numerous in Amoeba proteus than in Amoeba discoides. On the other hand, Sister Monica Taylor has often shown me specimens of Amoeba proteus containing very few and sometimes no crystals at all. A few years ago I carried out some experiments on the

crystals of Amoeba proteus, but I have arrived at no satisfactory conclusion about their formation or function. I think it certain that they grow in size and number as the amoeba grows in age. Schaeffer (1916) states that he has 'never with certainty been able to find any other form of crystal', i.e. the dipyramidal 'in this amoeba'. I have seen small cubic crystals in several specimens, but these cubic crystals are never as large as those seen in Amoeba proteus and their occurrence is much rarer. I have never seen in Amoeba discoides the large square plate-like crystals which occur in Amoeba proteus. Also in the cytoplasm of the young adult is a number of nutritive spheres. These are small (2 to 3μ), and of a definite greenish appearance. At this stage the nutritive spheres are quite structureless, and are not stained by Ehrlich's haematoxylin.

I have been able to confirm all Schaeffer's published observations on the living nucleus. It is normally single, disk-shaped with rounded edges, and with an average diameter of about 40μ by 15μ to 18μ thick. One nucleus was 56μ in diameter, though why it should be so much above the average size I am unable to say. The two larger surfaces are generally concave (Text-fig. 2 a). The surface is smooth, without folds. The nucleus is carried along in the flowing endoplasm, but generally holds a position about one-third the length of the amoeba from the posterior end. If and when it is carried nearer the anterior end it remains stationary while the cytoplasm flows over and round it, and thus it regains its normal position. While being carried along the nucleus is all the time rolling over and over. It is my opinion, however, that the nucleus of Amoeba discoides rolls over more quickly and consequently more often, and so one obtains an edge view or 'elevation' view much more frequently, than in Amoeba proteus. In rolling over the nucleus sometimes bends on itself and may remain so for some time, having thus the shape of a kidney-bean (Text-fig. 2 b), as observed by Schaeffer (1916). As the cytoplasm of Amoeba discoides is much less voluminous than that of Amoeba proteus, and as it spreads this smaller volume over a large surface area, the living nucleus is always visible, and

the chromatin masses under the nuclear membrane and in the karyosome are easily seen. The nucleus has a coarse mottled appearance. There appears to be a great deal of fluid of a mobile nature within the nucleus, and in this the karyosome changes position so that sometimes it is central and at other times lies to one side. Indeed one gets the impression that the karyosome itself contains mobile liquid readily changing position from one part of it to another as the nucleus itself is being rolled about
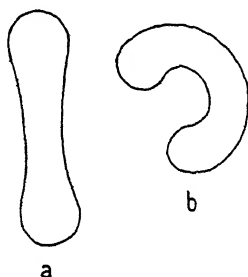
TEXT-FIG. 2.

Diagrammatic representation of the nucleus to show (a) 'elevation' view with rounded edge and concave faces. (b) the nucleus bent.

in the endoplasm. This hypothesis is confirmed by the variety of appearances which one comes across when studying a large number of fixed specimens. In many of the young adults the nucleus lies in clear cytoplasm, yet I am not prepared to call this clear space a vacuole. There does not seem to be any definite boundary line round it as there is, for instance, round the contractile vacuole. It seems to me to be a region of very clear and very mobile cytoplasm free from cytoplasmic inclusions, which gradually merges into the ordinary cytoplasm containing crystals, nutritive spheres, &c.

In most specimens there is a single contractile vacuole which reaches a diameter of 30μ. It is however quite a common thing to find two contractile vacuoles, a primary one at its maximum size and a secondary one beginning to grow. As the primary one bursts the secondary takes its place, while another secondary is formed at once. The contractile vacuole is generally formed

near the nucleus, and moves along with it until it is ready to burst when it lags behind the nucleus and takes up a position near the posterior end of the amoeba. In this position it bursts very slowly and very gently without causing any apparent disturbance within the cytoplasm or in the surrounding fluid. The rate of growth of the contractile vacuole is not regular, but I have observed it to be more rapid and more regular in young specimens. The irregularity may, of course, be due to the imprisoned conditions in which it is necessary to examine the amoebae.

Amoeba discoides feeds on Flagellates of various sizes, on green unicellular plants, and on Rotifers. When the latter are plentiful in the culture they generally form the staple food of the amoebae and it is quite a regular occurrence to find as many as nine while occasionally twelve Rotifers may be seen ingested by one individual amoeba. The variety and the amount of food which an individual amoeba can contain at the same time in its cytoplasm is truly amazing.

I have a culture at present (July 1937) in which, though it contains a plentiful supply of the above-named organisms, the amoebae have taken to ingesting long pieces of filamentous algae. Whether these latter are wholly digested or ejected undigested I am as yet unable to say.

DESCRIPTION OF THE AMOEBAE WHEN THE CULTURE IS BECOMING SENESCENT.

Viewed by reflected light the amoebae at this stage (i.e. old adults) are white and opaque. In transmitted light the crystals are seen to be large and very numerous. The nutritive spheres which at the beginning of the 'optimum' period were small and inconspicuous, are now large (5μ to 6μ) and exceedingly numerous, filling up much of the endoplasm. Their definite green appearance tints the whole Amoeba. The spheres tend to collect together into groups of from twenty to thirty, and such a group is often seen at the tip of a pseudopodium to the exclusion of crystals and other cytoplasmic inclusions (Text-fig. 3). Schaeffer (1916) was of the opinion that the number of these spheres which he designated 'so-called excretion spheres'

depended on the amount of food digested by the amoeba. I confirmed that observation in 1924 on A m o e b a p r o t e u s (see Taylor, 1924). Now, however, in the light of much more experience and intensive observation, I think that the amount of food eaten by the amoeba is not the only factor at work. To give but one out of many instances, in the early part of 1936, owing to stress of other work, my culture of A m o e b a d i s - c o i d e s was neglected. Whilst not actually starved, it was
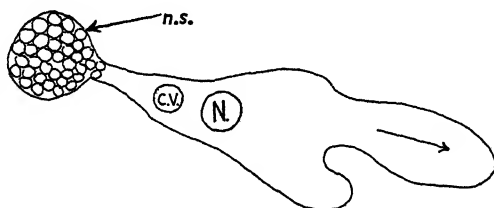
TEXT-FIG. 3.

Outline drawing of A m o e b a d i s c o i d e s (not to scale) to show how the nutritive spheres collect into a group at the end of a pseudopod. *n.s.*, nutritive spheres.

decidedly underfed, yet when I resumed work on it in July I found that all the amoebae were stuffed with very large nutritive spheres. These amoebae were then about six months old, and from that time onwards they began to disappear gradually from the culture.

The nutritive spheres grow in number and in size as the amoebae grow in age. When a culture is healthy and under- going its normal cycles the amoebae are always found to contain many large nutritive spheres just before the so-called 'depres- sion' periods. It has been shown for A m o e b a p r o t e u s (Taylor, 1924) that the nutritive spheres are intimately con- nected with the formation of encysted young amoebae. As will be shown later, I have had evidence of this being true also for A m o e b a d i s c o i d e s.

In April 1937 a culture of A m o e b a d i s c o i d e s reared in a Petri dish was in an especially flourishing condition, containing a very large number of adults. The food organisms however in this culture were sparse, and it was evident that in a short

time starvation conditions would prevail. Consequently a sub-culture was made by transferring a portion of the material (about one-tenth of the whole) to a Petri dish containing an excellent culture of food organisms, Rotifers, Flagellates, &c. Some food organisms were also added to the parent culture. On examining both cultures three months later (July 1937) their conditions were found to differ greatly. The parent culture contained no food organisms. The amoebae, though still plentiful, had become senescent—being black by transmitted light—full of crystals and large nutritive spheres—sluggish—refusing to grip the substratum and flow like healthy individuals. In the sub-culture which still contained a good supply of food organisms, the amoebae had multiplied to an extraordinary degree. They were in beautiful condition, and as soon as placed under the cover-slip they gripped the substratum and flowed actively. The crystals and nutritive spheres were small. The lack of food in the one case had brought on senescence. The plentiful supply of it in the other had warded off this condition, the amoebae being kept in good condition by repeated fission. However, this fission cycle will not continue indefinitely no matter how plentiful the food supply, and there is evidently still much work to be done on the nutrition of the amoebae.

Methods of Fixing and Staining.

For the purpose of examining the fixed and stained nucleus three types of preparations were made:

1. By means of a fine pipette a drop of the culture water containing the amoebae was put on to a glass slide and a cover-slip placed over it. The slide was then left to stand for a time, in a damp chamber, sometimes overnight, so as to give the amoebae time to settle down, grip the slide, assume the flowing state and stretch out to their full length. Aceto-carmine was then run under the cover-slip and the preparation thus made examined at once. Very many specimens fixed and stained by this method were examined.

2. The amoebae were put on to the slides and given time to settle, but instead of aceto-carmine Bouin's fluid was used to fix them. After fixation in Bouin the amoebae were washed,

stained in Ehrlich's haematoxylin, dehydrated, cleared in xylol and made permanent with Canada balsam, all by the irrigation method. Many beautiful preparations have been obtained by this method.

3. At times when the amoebae were very plentiful in the culture large numbers of them were put into a Petri dish. Then working under the low power of the binocular with the help of needles and a fine pipette the amoebae were freed as much as possible from the debris of the culture. This latter was as far as possible removed, as was also much of the supernatant water, leaving only just enough water to keep the amoebae 'happy'. The Petri dish was then flooded with Bouin's solution. The material thus fixed was pipetted into centrifuge tubes where the washing, staining, dehydration, and clearing was carried on. Finally, the amoebae were permanently mounted on slides in Canada balsam. Bouin's fluid proved an excellent fixative, and Ehrlich's haematoxylin as a stain leaves nothing to be desired.

DESCRIPTION OF THE FIXED AND STAINED NUCLEUS.

The fixed and stained nucleus varies in diameter from 21μ to 45μ, with an average diameter of about 36μ. It is surrounded by two membranes. Of these, the outer is very definite and sharply differentiated, looking like a distinct blue skin in the stained preparations. The inner membrane is much less definite. There is a clear space between the two membranes (figs. 3, 4, 7, 8, 11, Pl. 32). Immediately within the inner membrane, in fact close against it, lie the large chromatin blocks (figs. 2–12, Pl. 32). In optical sections of the nuclei these blocks appear very regularly arranged and equally spaced. There is generally a second layer of chromatin blocks within the outer one, occasionally there is a third such layer, but the blocks of these inner layers are not so regularly arranged as those of the outer. The chromatin blocks stain easily in Ehrlich's haematoxylin—each block standing out clear cut and richly coloured like fully differentiated chromatin, reminding the observer of a chromosome of a metazoan nucleus. The karyosome which carries masses of chromatin lies within the chromatin blocks region.

As a rule its diameter almost equals that of the nucleus. Sometimes, however, when the latter is seen in 'plan', the karyosome cannot be distinguished from the rest of the nucleus, which means that it extends right out to the inner nuclear membrane (fig. 6, Pl. 32). As has already been said in describing the living nucleus, the karyosome takes up various positions in the nucleus, and the stained preparations show that it often is much bent on itself. That the nucleus, and even the karyosome, contain much fluid also becomes evident from an examination of the stained preparations (figs. 3, 5, 6, 7, 11, Pl. 32). I think there can be little doubt that there is also a layer of fluid between the inner and outer nuclear membrane.

DIVISION OF THE NUCLEUS.

I have fixed, stained, mounted, and examined a very great number of Amoeba discoides at different times in the life of the culture. The beautifully expanded condition of the amoebae, and the details of the structure which can be seen in the organisms present in the food vacuoles are evidence that the fixation is absolutely satisfactory. Many of the nuclei examined are dividing—some just beginning to divide (figs. 5 and 6, Pl. 32), others further advanced in the process of division (figs. 8, 9, 10, 11, Pl. 32), but in no case is there any sign whatever of mitotic division. The division of the nucleus as a whole is amitotic, and so far as I can see a very simple, straightforward case of amitosis. The cleavage divides the nucleus as a whole as well as the karyosome into two parts. Each of these parts forms a new daughter nucleus. In one nucleus which had so divided, the two daughter parts had not separated from each other yet there was evidence of a second division taking place at right angles to the first. This observation was made on a temporary preparation in aceto-carmine, so I can only represent diagrammatically what I saw (Text-fig. 4). In Amoeba proteus it is possible to find four or even eight nuclei in individual amoebae. In the present investigation I found only one specimen in which one of the newly formed daughter nuclei had already divided into two before the cytoplasm showed any signs of division (fig. 11, Pl. 32).

OBSERVATIONS ON THE LIFE-HISTORY OF AMOEBA DISCOIDES.

So as to make quite clear what has to follow in this section I think it advisable to give at this point some readings from the record of the culture A.

14/11/34. The culture was started in the manner explained at the beginning of this paper (p. 461).

12/12/34. No adult amoebae could be found when the culture was examined with the low power of the Greenough
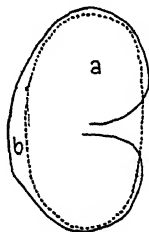
TEXT-FIG. 4.

The 'mother' nucleus had divided into two 'daughter' nuclei (a) and (b). (a) is shown by a broken line lying beneath (b). These two daughter nuclei had not separated yet (b) had begun to divide at right angles to the plane of the original division.

binocular. An examination by the ordinary microscope was not made.

11/3/35. The culture contained numerous adult amoebae some of which were yellowish in tint. A sub-culture made at this date did not for some unknown reason succeed.

23/7/35. Only one opaque adult was found after a careful search, but there were many very beautiful young amoebae. When these were put on to a slide in a drop of the culture water for examination they spread out quite readily and formed long pseudopodia (Free-hand outline sketches of these young amoebae are shown in Text-fig. 6, a and b). These young amoebae were feeding heavily on unicellular algae, this being the only available food at the time.

11/10/35. Many large adults were present. These when examined

with the microscope were found to be in an active healthy condition.

30/11/35. Amoebae were not nearly so plentiful as when last examined. It was evident that the culture was approaching a 'depression' period.

4/1/36. Very few adults could be found. Many young amoebae present, these were healthy looking and feeding heavily.

13/4/36. Adult amoebae were plentiful.

6/7/36. Adult amoebae plentiful, but very opaque when seen under the binocular with reflected light over a black background. When examined with the microscope the amoebae were found to be stuffed with crystals and very large nutritive spheres. As these amoebae were about six months old, the culture was carefully watched during the next few weeks. The number of adult amoebae grew less and less, but before they had all finally disappeared the culture contained numbers of micro-amoebae. These micro-amoebae will be described later.

(Note.—The term micro-amoeba is used for the small amoebae recently emerged from the cyst and undergoing development.)

From a study of this record it is evident that, as has already been said, there are times when the culture contains no adult amoebae but such times are only so-called 'depression' periods for during them numbers of micro-amoebae can be found. These young amoebae grow up and in their turn increase in number by fission.

The question now to be considered is the formation of these young amoebae, and though I have not yet seen all the stages described for Amoeba proteus (see Taylor, 1924), those I have seen are, I think, sufficient to prove that agamontogony in Amoeba discoides is similar to that in Amoeba proteus.

The 'old' adult with its large nutritive spheres is the agamont. When an agamont has been fixed and stained in Ehrlich's haematoxylin it becomes evident that there are two types of spheres in its cytoplasm—the ordinary large deeply stained nutritive spheres and other still larger spheres which are

definitely differentiated into palely stained and deeply stained regions. These larger spheres are the developing agametes. In the process of differentiation they use up the nutrient material of the nutritive spheres which latter in consequence lose their staining capacity. In a fully formed agamete it is possible to distinguish the new cytoplasm which is pale-staining from the chromatin which stains more deeply (fig. 13, Pl. 32). Dr. Monica Taylor (1924) has shown how in Amoeba proteus the chromatin blocks escape from the nucleus and become associated with the nutritive spheres to form the rudiment of the agametes.

I have not seen the chromatin blocks actually escaping from the nucleus of Amoeba discoides. I have, however, found specimens, containing fully differentiated agametes, in which the nucleus had no visible membrane (fig. 12, Pl. 32). Now ordinarily the nuclear membrane is a conspicuous structure which stains very readily. It seems reasonable then to conclude that in these specimens the nuclear membrane has dissolved in order to set the chromatin blocks free into the surrounding cytoplasm. Here these masses of chromatin uniting with the nutritive spheres differentiate round themselves a certain amount of cytoplasm and become fully formed agametes (fig. 13, Pl. 32). When the agamont disintegrates the agametes are set free into the culture water where they remain in an inactive, encysted condition for a varying period of time (fig. 14 a–g, Pl. 32). Finally, the little amoebae escape from the cysts. At first these micro-amoebae are circular in general outline, from 20μ to 25μ in diameter, with a great number of very short blunt pseudopodia radiating in all directions (Text-fig. 5, a and b). The cytoplasm is clear and streams very slowly, so that there is little change in the creature's position on the slide. The nucleus is very conspicuous, the pale green-looking karyosome standing out clearly, the rest of the nucleus forming a clear zone round it. At this stage the young nucleus of the micro-amoeba does not roll over in the cytoplasm, it is always seen in 'plan'. The contractile vacuole which lies beside the nucleus is also a conspicuous feature. It grows rapidly, bursts regularly and very gently. Many of those micro-amoebae contain no food

vacuoles while others are stuffed with them, the former, I conclude, being those most recently emerged from the cysts.

In the next stage the blunt pseudopodia have been withdrawn and replaced by a single large pseudopod so that the amoebae
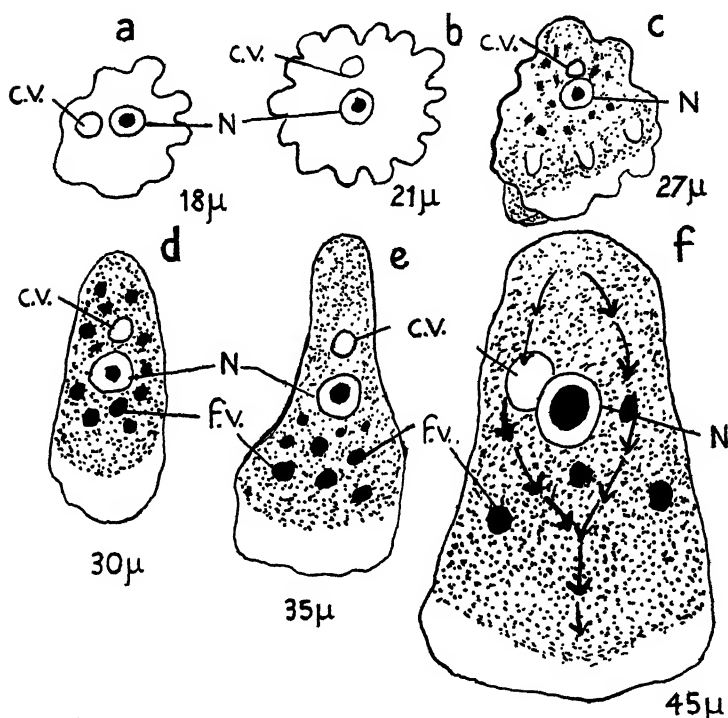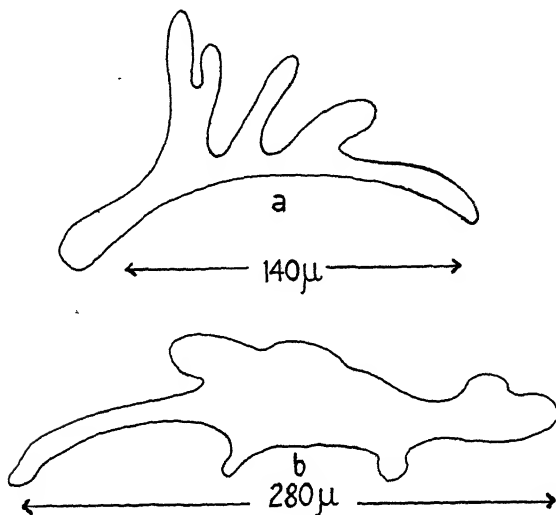

TEXT-FIG. 5.

Free-hand drawings of very young Amoeba discoides to show the gradual change in shape as they grow older. The drawings are not to scale, but the amoebae were measured and the greatest diameter of *a*, *b*, and *c* and greatest length of *d*, *e*, and *f*, given in μ. *N*., nucleus; *c.v.*, contractile vacuole; *f.v.*, food vacuole.

are no longer circular but oblong in outline, about 30μ to 40μ long (Text-fig. 5 *d*, *e*, *f*). A great part of this pseudopodium—the anterior portion of it—consists entirely of clear cytoplasm (Text-fig. 5 *d*, *e*, *f*). The cytoplasm flows rapidly so that the

little creature travels along at a considerable rate. The nucleus which is very conspicuous, and still seen always in plan, seems to act as a dividing centre for the cytoplasm which flows round it in two streams, one on either side (Text-fig. 5 *f*). The contractile vacuole beside the nucleus behaves as in the earlier

Text-fig. 6.

Outline drawings of two young Amoeba discoides when they have become adult in form. The figures are not drawn to scale but the longest axis as measured in μ is indicated.

stage, while the food vacuoles are larger and more numerous than they were in that stage.

After this the amoebae begin to look much more like the adults (Text-fig. 6 *a* and *b*). The single pseudopodium which consisted mostly of clear cytoplasm being replaced by many pseudopodia in which the endoplasm is more granular. The tips of the pseudopodia are always, at every stage of development as well as in the fully grown adult, blunt, that is, rounded.

Summary.

1. A large free-living amoeba found by Mr. Harry Watkinson in the tropical fish tanks of Mr. Albert Sutcliffe of Grimsby has

been identified as Amoeba discoides (Schaeffer, 1916) = Metachaos discoides (Schaeffer, 1926).

2. From the inoculation material obtained from these tanks Amoeba discoides has been successfully cultivated in the Notre Dame Training College Laboratory by a technique similar to that used for the cultivation of Amoeba proteus: wheat being the pabulum employed. In contrast to what obtains in the cultivation of Amoeba proteus, however, Amoeba discoides flourishes more luxuriantly in shallow Petri dishes, than in deeper troughs.

3. The nucleus in the resting and dividing stages is described; division is amitotic.

4. The more important cytoplasmic contents, including nutritive spheres, and crystals are likewise described.

5. The life-history has been worked out. The adult amoeba becomes an agamont giving rise to agametes which eventually grow into adult amoebae, the life-cycle occupying roughly about four months.

6. Descriptions of the nucleus of the newly hatched and developing amoebae are deferred.

I wish to offer my sincerest thanks to Professor Graham Kerr under whom this work was begun, and who has continued from afar to watch over it with ever kindly interest and encouragement and who has read the paper in typescript.

My thanks are also extended to Professor Hindle, under whom the work was completed, for his kind advice and for reading the paper in typescript.

In conclusion I would like to express my appreciation of her skill and of the care and trouble bestowed by Miss Brown Kelly in the execution of the original drawing of fig. 1, Pl. 31.

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EXPLANATION OF PLATES 31 AND 32.

LETTERING.

N., nucleus; *c.b.*, chromatin blocks of the nucleus; *C.V.*, contractile vacuole; *F.V.*, food vacuole (the organism being a large encysted Flagellate); *F.r.*, food vacuole (small Flagellates); *s.c.*, cubic crystal; *c.*, dipyramidal or oval crystal; *N.S.*, nutritive spheres; *k.*, karyosome; *n.m.*, nuclear membrane; *n.sp.*, nuclear sap; *n.s.*, nutritive sphere; *ch.*, chromatin in the karyosome.

PLATE I.

Fig. 1.—Free-hand drawing (not to scale) made from a large, living adult Amoeba discoides.

PLATE II.

All the figures were drawn from specimens fixed in Bouin's fluid and stained in Ehrlich's haematoxylin. Camera lucida with a No. 5. compens.-ocular and a Zeiss Apochromat. 2 mm. oil immersion objective.

Figs. 2, 3, and 4 represent resting nuclei; the karyosome of 2 is seen in 'plan'; of 3 in 'elevation', and of 4 pushed to one side of the nucleus.

Figs. 5 and 6 early stages of division of the nucleus.

Fig. 7.—Nucleus dividing and bent into a figure of eight shape.

Fig. 8.—Division of the nucleus almost complete, half the karyosome passing into each daughter nucleus.

Figs. 9 and 10.—The nuclear division is completed, but the daughter nuclei have not separated.

Fig. 11.—Three nuclei from one amoeba. Cytoplasmic division has not kept pace with nuclear division for one of the daughter nuclei of the first division has already divided.

Fig. 12.—A nucleus with no trace of a nuclear membrane.

Fig. 13.—Small portion of an amoeba—an agamont showing nucleus without nuclear membrane; *a*, agametes and n.s., ordinary nutritive spheres.

Fig. 14.—Encysted agametes, *a–g*, after disintegration of the agamont.
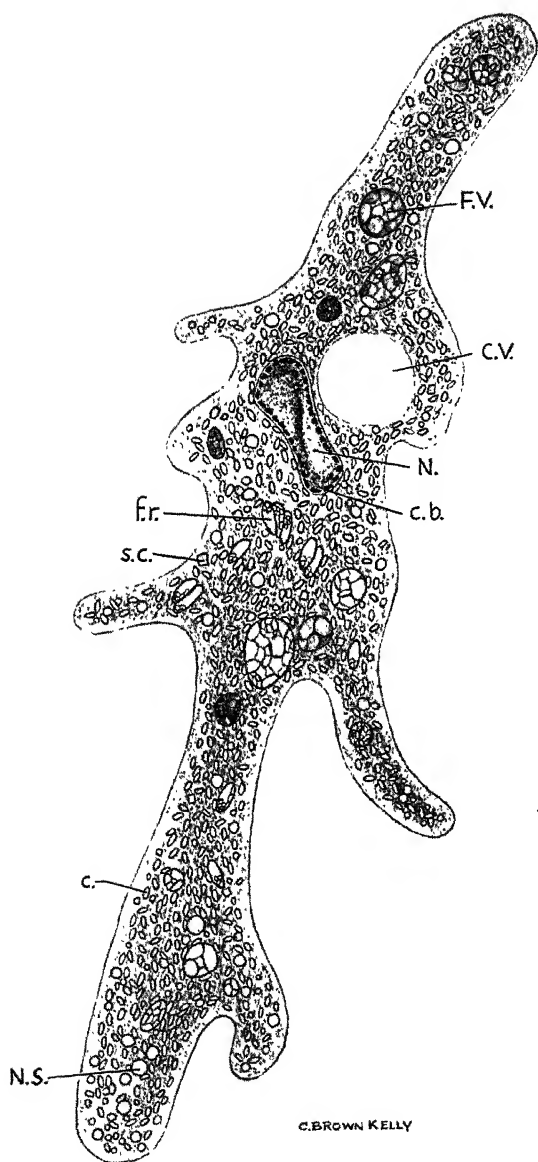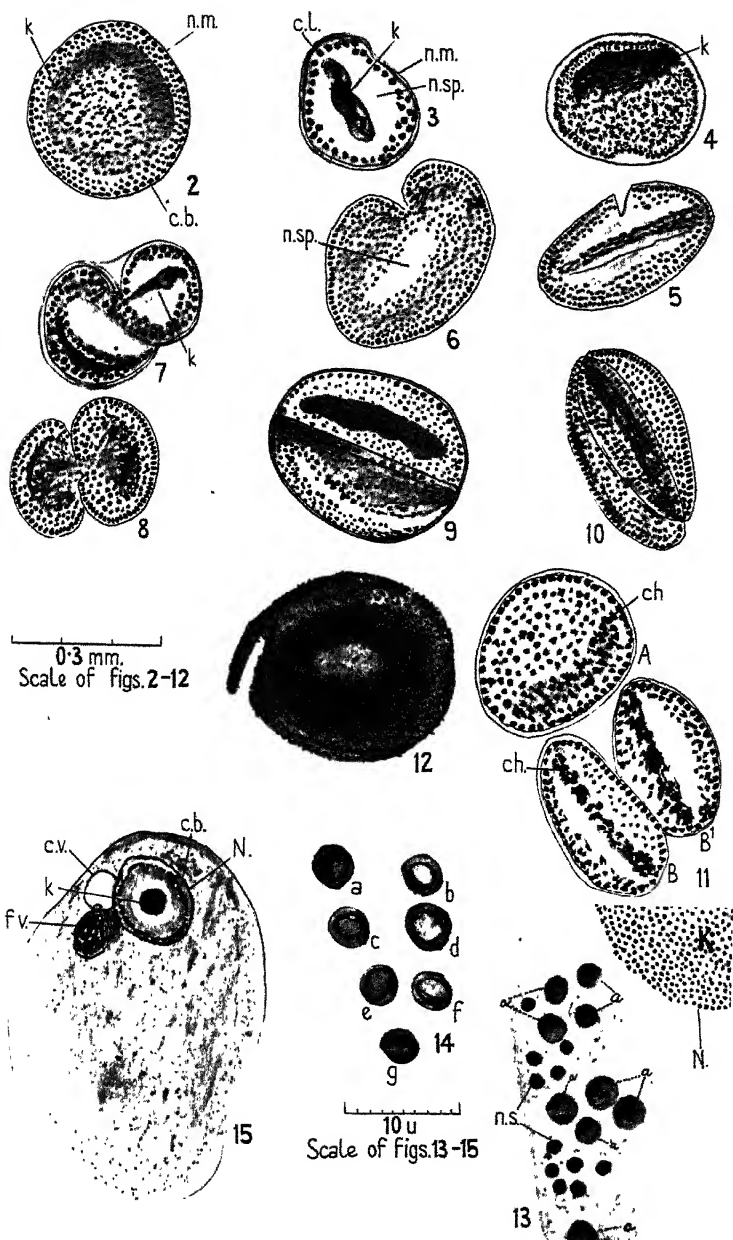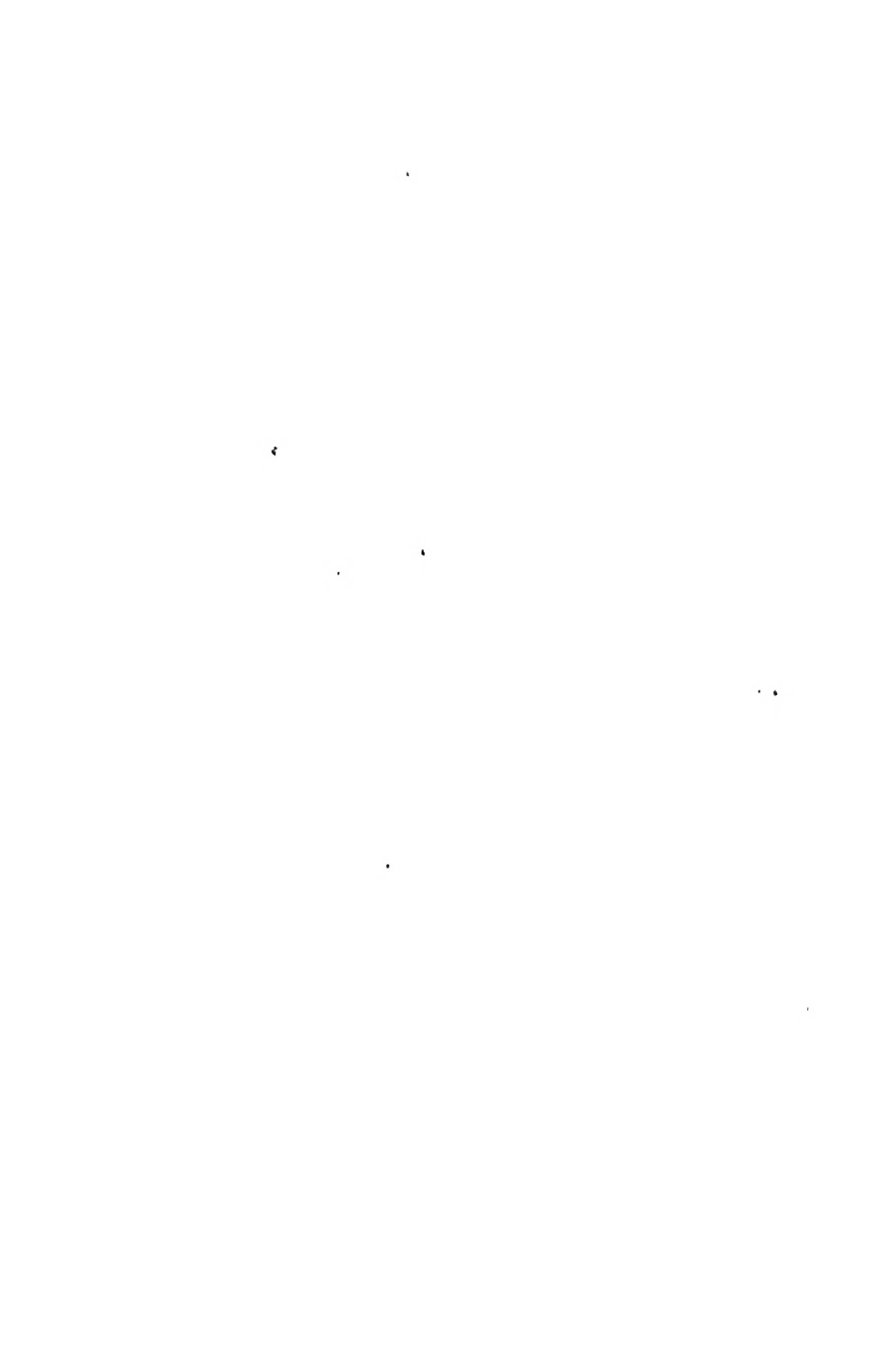
Fig. 15.—A young Amoeba discoides.

F.V.

C.V.

N.

f.r.

c.b.

s.c.

c.

N.S.

C.BROWN KELLY

Fig. 1

0·3 mm.
Scale of figs. 2-12

10 u
Scale of figs. 13-15

Structural Changes in the Anuran Mandibular Arch during Metamorphosis, with reference to Rana Temporaria.[1]

By

H. K. Pusey, M.A.

Assistant Lecturer, Department of Zoology, University College, London.

With Plates 33 to 45.

Contents.

[1] The substance of this paper was written in June 1936 and was successfully submitted as a thesis for the degree of Bachelor of Science in the University of Oxford.

1. Former Work and the Problem.

Very many workers have undertaken investigations on various points of cranial morphology in the Anura. Of these, for the present purpose, Gaupp (1893 and 1906) is by far the most outstanding. Since Gaupp published his most excellent monograph in 1893, no other worker has investigated the problems of jaw metamorphosis in such detail. Later work dealing with the jaws has been related rather to muscles than to cartilages (Luther, 1914; Litzelmann, 1923). Passing reference has been made to jaw anatomy and metamorphosis by a very large number of other workers, but the whole matter is so complicated by the relative movement of the parts, by the destruction of pre-existing structures, and by the rapid growth and movement of new structures, that only a detailed stage-by-stage study is effective in explaining them. The study of sections unaided by reconstructions is not an effective method of attack, and the manufacture of solid reconstructions must be hampered by the very vague outlines shown by those cartilages which are undergoing destruction. Consequently the description of the jaws during the metamorphosis has not been advanced in any detailed way since 1893.

No attempt will be made to sum up or to assess the value of work done prior to 1893; this labour has been fully and satisfactorily undertaken by Gaupp. Reference to more recent work will be made as required in the discussions at the end.

Gaupp's paper (1893) was very detailed, and I have found his observation of fact to have been extremely accurate as regards the jaws; I have detected no major errors, and have found very little to correct even in the details. His account is least full at the time of the fusion of the quadrate to the neurocranium by the otic and pseudobasal processes; I have tried to make good this deficiency.

The description of jaw metamorphosis given here is taken purely from my own investigations and is related particularly to the reconstructions shown in the figures. The work was undertaken independently of the accounts given by Gaupp and was intended to serve as a check on them. The two accounts are so similar that it would be wearisome to make continual acknowledgement of Gaupp's findings; only occasionally will such reference be made. My own account is somewhat more complete in certain details of the destruction and buckling of the quadrate, and of its re-attachment in the temporal region. But though Gaupp's account is very excellent and can easily be understood by a reader who is familiar with the details of the jaw metamorphosis and who has sections and reconstructions available for study, it becomes somewhat involved for one not so equipped. The chief criticism to be brought against his paper is the scanty use he makes of figures. Such figures as he gives are frequently oblique views of somewhat isolated parts of the skull or jaws, and are therefore not easily comparable one with another. It is to be hoped that these defects are absent from the present series of figures.

But though Gaupp's description of the facts is so thorough and so reliable, his interpretation of those facts has now become somewhat out of date, and even at the time of publishing was unable to account for the homology of certain structures. More recent work has made a reinterpretation imperative. A scheme of homologies has been devised in this paper, which takes account of all the structures concerned and relates them to structures found in other frogs and in the Urodeles. The scheme must, however, remain in part hypothetical until further work has been undertaken, particularly on the development of the primitive frog Ascaphus. It may be pointed out at once that the view here adopted is at variance in certain points from the newly developed theory of the hyostylic attachment of the Anuran jaws (Kruijtzer, 1931), and in these points contains new—though as yet unproved—suggestions.

When Gaupp published his paper he was still unable to find a homologue in the Urodela for his Anuran 'commissura quadrato-cranialis anterior'; in addition, he was faced with the

problem of the possession by the frogs of separate larval and adult otic processes; whilst he disposed of the tadpole 'muscular process' by saying that it was a larval specialization unrepresented in the Urodela. More recently his interpretation of the 'basal articulation' has been called in question by de Beer (1926). Very recently, too, the primitive frogs Ascaphus and Liopelma have been described by de Villiers, and by Wagner; the descriptions given raise new problems. Whilst finally the views supported by Kruijtzer call for further investigation in the light of this newest work.

The main problems to be answered then seem to be:

1. What is the homology of the 'commissura quadrato-cranialis anterior'?
2. What light can be thrown on the possession by the frogs during development of two otic processes and a muscular process?
3. What is the true nature of the 'pseudobasal' articulation?
4. What was the structure of the temporal region of the skull of the ancestral Anuran?

In addition, the present paper sets out to give a more detailed account, with figures, of the jaw metamorphosis. An attempt is also made to see how far the evolution of the larval jaw arrangement can be explained from comparative anatomy, and to give an interpretation of the metamorphic changes.

2. ACKNOWLEDGEMENTS.

checking certain facts and corrections on material in the University Department of Zoology; and Miss Joyce Townend for advice on the preparation of the figures.

3. The Nomenclature and Homologies of certain Cranio-quadrate Articulations employed by former Workers.

The interpretation of the attachments of the Anuran quadrate to the skull has long been obscure; the obscurity was at first due as much to confusion in the use of nomenclature as to inaccuracy in observation. An attempt will, therefore, be made to follow through the names used by earlier workers and to relate them to the names at present in use; in this way it is hoped that a new value may be added to this earlier work.

In his famous Croonian lecture in 1858 Huxley introduced the word 'suspensorium' as the name for a c o m m o n attachment of both the mandibular and the hyoid arches to the skull. In the frog-tadpole he then stated that the tadpole otic process was the 'whole suspensorium, and not merely the quadratum of the branchiate vertebrate'. He thus introduced the erroneous idea of a hyoid element in this structure in the tadpole. He noted correctly the ascending process and the commissura quadrato-cranialis anterior but gave them no definite names.

Parker, in 1871, followed Huxley's false lead and in addition made some original wrong observations. He believed that the upper end of the hyoid arch was fused on to the upper end of the mandibular arch (tadpole quadrate cartilage) behind it. This, in fact, is quite untrue. It led him to call the tadpole otic process the 'supra-hyomandibular'. He noted (1) the knob on the quadrate behind the ceratohyal; (2) apparently in later stages, the debris of the folded quadrate bar; and (3) in later stages again, the pseudobasal process. In their respective stages he named all these structures 'infra-hyomandibular'. The lower end of his supposedly fused 'hyomandibular' he called 'symplectic bar'; this was the 'posterior spur of the quadrate' of this paper.

He had also seen the ascending process which he rightly believed to be of mandibular origin; this he variously named the 'metapterygoid connective or root', or 'mandibular root'.

In 1874 Huxley examined Menobranchus (= Necturus), and compared it with the frog. He noted the ascending process, and gave its true relations to the branches of the Vth nerve; he called the basal process of the quadrate the 'pedicle of the suspensorium', and he noted the otic process and described the relations of the branches of the VIIth nerve. But in his comparison of this Urodele with the frog he made a number of mistakes. In the tadpole he described the ascending process and homologized it with the 'pedicle' of Menobranchus, thus making it a basal process, which is wrong; he even stated that its relations to the branches of the Vth nerve were the same as in the Urodele!

In the metamorphosis of the frog he believed that his 'pedicle of the suspensorium' (correctly, the ascending process) was carried outwards to become the inner attachment of the adult (the pseudobasal process). This is not the case, as the ascending process is wholly destroyed; but in this way, by attributing to it an origin from the ascending process, he made the pseudobasal process of the frog also the homologue of the basal process of the Urodele, an error which persisted far into the present century, with support from Gaupp (1893 and 1906). He described the frog's otic process. He was left to find in the frog a homologue of the Urodele ascending process, and in the Urodele a homologue of the tadpole 'orbitar process' (muscular process). He was unable to meet this demand in view of his previous statements.

In the same year (1874) Huxley published his researches on the columella auris. The findings of this investigation allowed him to correct Parker's error of 1871 which had sprung from his own error expressed in his Croonian lecture. He now showed that the mandibular arch (quadrate cartilage) of the tadpole contained no fused hyoid element. In consequence he was able to rename Parker's 'supra-hyomandibular' as an 'otic process', and show it to be homologous with that of the Urodeles.

In 1876, in his second paper on the skull in the frogs, Parker admitted Huxley's correction, and changed his 'supra-hyomandibular' to 'otic process', but introduced a new error by saying that it was separated off to give the 'annulus tympanicus';

(in 1871 he believed that it gave the columella cartilages of the adult).

He believed that the ascending process—now his 'pedicle', following Huxley's error—remained intact into the adult in the toad (Bufo vulgaris), whilst in other frogs its outer end only remained, and developed a 'condyle of the pedicle' (the pseudobasal process) to abut against the auditory capsule; he thus assumed that the pseudobasal process was of quadrate origin. Parker was therefore nearer to the truth than Huxley, in that he admitted the (partial) destruction of the pedicle (ascending process) and looked on the 'condyle of the pedicle' (the pseudobasal process) as a new growth; but he was wrong in believing it to be an outgrowth of the ascending process or of the quadrate at all. Parker states these views more shortly in his book on the Skull written jointly with Bettany, 1877.

Huxley (1876) in a paper on Ceratodus confirmed the use of the name 'pedicle' for the basal process of the quadrate. In the same year Parker, in his paper on Urodeles I, made clear his views on the homologies of the quadrate attachments in Urodeles and frogs. In the Urodele he recognized an otic, and an ascending process and also a pedicle (true basal process). But he went on to say that this 'pedicle' is the homologue of the frog's pedicle which by description is the frog's ascending process. And he was then compelled to say that the frog had no ascending process. This is exactly Huxley's view in 1874.

Stöhr in 1879 gave a correct description of the otic, ascending and basal processes in the Urodela and, in 1881, was the first worker to realize that the 'pedicle' of the Anuran (i.e. its ascending process) was the homologue of the ascending process of the Urodela.

These continual errors in homology, coupled with a somewhat loose use of terms, and very real errors in the interpretation of the facts of development, greatly detract from the clarity and usefulness of the earlier work.

Gaupp in 1893 made a very exhaustive investigation of the frog's chondrocranium and jaws; he reviewed work done up to that date, and corrected the errors of fact. But his system

of homologies for the cranio-quadrate attachments seems to the present writer not to be entirely satisfactory. However, he described a tadpole and an adult-otic process, the ascending process, and the 'basal process', in addition to other attachments with which we are not immediately concerned. He believed that the basal process was the homologue of the structure of similar name in the U r o d e l e s—this may possibly still prove to be true, though it is not the view put forward in this paper. But his implied suggestion that the attachment of this process to the auditory capsule is homologous with that of the U r o d e l e is certainly wrong. However, his view has been accepted since by Edgeworth (1925) and other workers (de Villiers and his pupils), at least to the extent that they use his nomenclature for naming these structures.

In 1926 (pp. 310 and 336), however, de Beer pointed out that the articulation of this 'basal process' with the auditory capsule was not similar to that of U r o d e l e s; he explained the differences in the relations of the branches of the VIIth nerve in the two groups and he used the word 'pseudobasal' to describe this articulation. Nevertheless, he followed Gaupp in attributing a quadrate origin to the 'basal' process and retained the name 'basal' to describe it. Goodrich (1909, fig. 59 B) had already figured this articulation and showed the correct relations of the VIIth nerve to it, but he made no comment in the text.

In this paper an attempt will be made to show that this 'basal process' is not a process of the quadrate at all, but is rather to be considered either as the isolated end of the (cranial) 'basitrabecular process', or as a forward outgrowth of the (cranial) 'post-palatine commissure'. Other recent workers (Kruijtzer, 1931) have put a different interpretation on the facts and would return in effect to the standpoint of Parker (1871) with his 'supra- and infra-hyomandibular'. The long story of the attempts to explain this process is therefore probably not yet at an end.

4. MATERIAL, METHODS, AND FIGURES.

The tadpoles used were taken during three seasons as spawn or newly hatched larvae from water near Oxford. They were

reared in tanks in the laboratory and were fed on a liberal mixed diet of animal and vegetable food and on Drosophila after metamorphosis. Samples from the stocks were fixed daily; some in 4 per cent. formol, some in corrosive formol, and some in Bouin's fixative; those fixed in corrosive formol were subsequently treated with iodine solution and acid alcohol. In addition, young frog stages were captured in the wild after metamorphosis and were fixed similarly.

The material was studied by three methods:

1. In serial sections cut at 15 or 20μ both transversely and sagittally for each stage. Borax carmine was usually used as a bulk stain (though sometimes omitted); and this was followed by picro-nigrosin.

2. Dissections of whole chondrocrania were made by van Wijhe's method, using victoria blue (van Wijhe, 1902 and 1922).

3. Alizarin transparencies were made by methods described by Gray (1929), for the study of bone development.

Victoria blue preparations proved useful in showing the general changes in the jaws as metamorphosis proceeds, and they were used as a check on the proportions of the reconstructions made from sections. However, the dye failed to stain either young or degenerate cartilage, so that the resulting picture obtained from the preparations was necessarily incomplete, and to this extent proved most misleading in the interpretation of sections. Sometimes the dye failed to stain any cartilage at all (compare van Seters, 1922).

To overcome this difficulty two dimensional reconstructions were made of seven stages. For each stage a series of pictures was drawn with the aid of a microprojector at a magnification of $33 \times$ linear, from sagittal sections. Direct composite tracings from these pictures gave accurate 'contour-line reconstructions'. Such formed the basis of figs. 1 to 12, and 20, 23, and 24. Using the same series of pictures it was possible, by a projection method, to produce the dorsal and anterior views shown in figs. 13 to 19, and 21, 22, 25, and 31. Figs. 26 to 30 were made by the contour-line method from the published drawings of de Villiers, Wagner, and van Seters. The writer takes full responsibility for any errors which may have crept into these

last five pictures on account of the small number of original drawings which could be combined to produce them. Miss de Vos, of Stellenbosch, very kindly sent me two camera lucida drawings from the series of Ascaphus sections worked on by de Villiers, which represent sections in advance of those figured in his paper. Drawings from both sources were combined to give figs. 26 and 27.

The descriptions which follow are based on the study of some fifty animals prepared by the various methods given above. In addition, use was made of sections of Rana, and of wax plate reconstructions made by the late Dr. J. W. Jenkinson, which are now in the collections of the Department of Zoology and Comparative Anatomy, Oxford.

The writer hopes to publish the details of his methods of reconstruction as a separate paper.

5. The Description of Seven Developmental Stages—Cartilage only.

Stage I (fig. 1, Pl. 33; fig. 8, Pl. 36; fig. 17, Pl. 39).

External appearance: Length of body 13 mm.; length of tail 23 mm.; hind legs less than 1 cm.; front legs hidden; horny teeth present on jaws and 'lips'.

The quadrate cartilage (*q.*) of the tadpole skull is a long flat strap of cartilage which lies laterally to the cranial floor; it reaches from the anterior third of the auditory capsule almost to the front of the snout and slopes downward as it passes forward, so that its anterior end is below the level of the cranial floor (fig. 1, Pl. 33; fig. 8, Pl. 36). It is also inclined at an angle so that its upper surface faces obliquely inward toward the middle line. Behind, it is anchored to the chondrocranium by two attachments, the otic process and the ascending process. The otic process (fig. 1, Pl. 33, *pot.*, and fig. 17, Pl. 39, *pot.*) is a small rod of cartilage which projects forwards, outwards, and downwards from the wall of the lateral semicircular canal (*lc.*) of the auditory capsule. It is completely fused at its two ends, above, to the auditory capsule, and below, to the outer edge of the

quadrate cartilage (q.), and is thickest at its hinder end. Opposite the otic process the quadrate cartilage is continued inwards on its inner, hinder corner, as the ascending process (pa.) which is a rod of circular section sloping gently upward, as it passes to the wall of the neurocranium. It is completely fused with the trabecular region of the skull immediately behind and a little below the foramen for the oculomotor nerve ($focn$.). Its root thus lies across the front of the vertically elongated foramen pro-oticum (fp.) in such a way that nerves V_2 and V_3 pass out of the foramen above and laterally to it, while V_1 and VII palatine pass forward, below, and medially to it; VII hyomandibular passes out laterally behind and below it. The outer end of the process and the back of the quadrate bar lie across the front face of the auditory capsule, but there is no connexion between these structures until the otic process is reached at the side.

The quadrate cartilage bows out laterally as it passes forward from the auditory capsule (fig. 17, Pl. 39) and then swings back again towards the middle line to the articular region (paq.). Here its inner border is suspended to the neurocranium by the 'commissura quadrato-cranialis anterior' (Gaupp's nomenclature) (fig. 8, Pl. 36, cqa., and fig. 17, Pl. 39, cqa.). This is a flattened band of cartilage which sweeps steeply upward and inward to fuse completely with the neurocranial wall along its ventro-lateral edge, immediately behind the root of the trabecular horn (ct.). At this stage of development, as at earlier stages, the commissura passes backwards as it passes inwards (fig. 8, Pl. 36); this backward slope is more marked in younger animals. The base of attachment of the commissura to the trabecula is very broad, so that its hind border slowly curves backward into the subocular shelf (fig. 8, Pl. 36, sst., and fig. 17, Pl. 39, sst.). This shelf projects outwards and downwards from the side of the trabecula; in front it is sharp-edged laterally, but it becomes blunter on passing back and it finally disappears in the region below the optic foramen (fig. 8, Pl. 36, fon.); however, even after this point, the trabecula continues to project beyond the plane of the side wall of the cranium above it, as a flat-faced ledge which is continued backward and is

finally obliterated, as such, by the fusion of the ascending process with it.

In the angle formed by the commissura and the subocular ledge behind it, we find a flattened triangular cartilage projecting backward into the subocular space; Gaupp has called this the 'processus pseudo-pterygoideus' (fig. 8, Pl. 36, *ppq.*, and fig. 17, Pl. 39, *ppq.*). It is very variable in its degree of development in different animals of an otherwise similar stage and seems sometimes to be absent altogether.

The 'processus quadrato-ethmoidalis' (of Gaupp) lies opposite the processus pseudo pterygoideus but at a lower level and on the front edge of the commissura (fig. 1, Pl. 33, fig. 8, Pl. 36, and fig. 17, Pl. 39, *pqe.*). It projects forwards, upwards, and inwards, and is continued by means of the 'ligamentum quadrato-ethmoidale' (of Gaupp) (fig. 8, Pl. 36, *lq.*) to the side of the trabecular horn; the ligament embraces the tip of the process and is continued backward along the inner edge of the quadrate bar down to the pars articularis (*paq.*) as a fibrous band of tissue (the future pterygoid bone). Together the process and the ligament make a lateral and anterior border to the inner nostril which opens from the nasal sac into the mouth cavity; the trabecular horn and the commissura make the inner and hinder border of the nostril. Below the processus quadrato-ethmoidalis, the front edge of the commissura passes downward to the inner angle of the pars articularis of the quadrate (fig. 17, Pl. 39, *paq.*).

The inner corner of the pars articularis is the lowest point of the quadrate cartilage. From it the front border of the quadrate slopes upwards somewhat steeply, and at the same time passes forwards (fig. 8, Pl. 36, and fig. 17, Pl. 39) to its most anterior position; then, from here, again passes backward. Only the inner side of the spear-head so formed is the surface of articulation for the lower jaw. From the tip of the spear a ligament runs forwards, upwards, and inwards to the tip of the trabecula horn (fig. 8, Pl. 36, *tql.*); it has not been described before and it may be called the 'trabecular-quadrate ligament'.

Exactly opposite the commissura the outer edge of the quadrate cartilage is carried up into a high triangular 'processus

muscularis' (Gaupp) (fig. 1, Pl. 33, *pmq.*, and fig. 17, Pl. 39, *pmq.*)
It is a thin flange of cartilage with slightly thickened, out-
turned edges; it leans inwards and somewhat forwards (fig. 17,
Pl. 39) and is concave outwards. Its upper end is supported
to the commissura by loose fibrous strands. These and the
muscular process complete the roof of a tunnel whose floor is
the quadrate cartilage, and whose inner and outer walls are
the commissura and muscular process respectively. Through
this tunnel the mandibular muscles, nerves, and blood-vessels
pass to the jaw region in front.

Finally, the quadrate cartilage is notched below the hinder
part of the muscular process to give a surface for the articulation
of the ceratohyal (*ch.*) of the branchial apparatus (fig. 1, Pl. 33,
nc.). In the region of this notch the quadrate is narrowest from
side to side and is thin in section, whilst just in front it is
thickened (fig. 8, Pl. 36); these are points of importance in the
subsequent buckling of the quadrate bar, for this notch supplies
a point of weakness.

There are, then, three c a r t i l a g e attachments of the
quadrate to the skull in the tadpole, (1) the commissura in
front, (2) the ascending process medially behind, and (3) the
(tadpole) otic process laterally behind. In addition it is held
by two ligaments to the trabecular horn, (1) the ligamentum
quadrato-ethmoidale, and (2) the trabecular-quadrate ligament.

In the tadpole the whole of the quadrate bar lies behind the
pars articularis and the lower jaw; consequently it does not
supply the cartilage skeleton of the upper jaw. This deficiency
is made good by the specialization of the trabecular horn and
its apparent derivative, the supra-rostral cartilage (fig. 1,
Pl. 33, *cls.*, and fig. 17, Pl. 39, *cls.*) (= cartilago labialis superior,
of Gaupp). The trabecular horn (fig. 1, Pl. 33, *ct.*, fig. 8, Pl. 36,
fig. 17, Pl. 39, *ct.*) is a long flat strap of cartilage diverging gently
from its fellow and projecting forward from the cranial floor.
It expands slightly as it passes forward, and its outer end is
curved downward and is squarely truncated. The straight outer
ends of the two horns supply surfaces for the articulation of the
upper edge of the supra-rostral cartilage. This latter cartilage
(*cls.*) is crescentic in form and deep dorso-ventrally; there is

a V-shaped notch in its upper border medially (fig. 17, Pl. 39), while at the sides the horns of the crescent are obliquely cut away from below and behind (fig. 1, Pl. 33). It is supplied by a mandibular muscle, and it is opened by means of ligaments from the lower jaw; so that it is movable within limits on the ends of the trabecular horns, and acts as the functional upper jaw. Its lower edge, particularly on its inner, posterior side, is shod by a single crescentic horny tooth blade, formed by the cornification of the overlying epidermal tissue.

The lower jaw is made up of t w o elements on each side; these will here be called the anterior and posterior jaw cartilages (cartilago labialis inferior and cartilago Meckelii of Gaupp). The four elements comprising the entire mandible are set t r a n s - v e r s e l y across the snout in the form of a depressed $\diagup\diagup\diagdown$, the anterior cartilages lying in front of the posterior ones. The anterior cartilages (fig. 1, Pl. 33, *ajc.*, and fig. 17, Pl. 39, *ajc.*) are rectangular blocks, somewhat curved, with their convexity directed forward; they abut against one another medially with square-cut inner ends (fig. 17, Pl. 39) and are held together by a copula—a ball of tissue whose histological character is that of young cartilage; fibrous strands are also present. At the outer ends the ventral posterior corners are recurved and fit into sockets on the anterior faces of the posterior jaw cartilages. These posterior elements (fig. 1, Pl. 33, *pjc.*, and fig. 17, Pl. 39, *pjc.*) are of more complicated form. They are concave forward (fig. 17, Pl. 39) and have a slight sigmoid curvature when seen from in front. The front face of the inner end is cupped and receives the posterior corner of the anterior cartilage, while the lateral end is hooked. The hook fits over the pars articularis so that the outer end lies below it, while the remainder of the cartilage passes upwards and inwards, across the articulation; more medially the cartilage rises upward somewhat more rapidly to complete the S bend begun at its outer end (fig. 1, Pl. 33). The posterior cartilage is supplied with mandibular and hyoid muscles which shut and open the mouth, and, in addition, it is connected to the anterior cartilage, and to the supra-rostral, by ligaments. A single U-shaped horny tooth blade is bound on to the front faces of the anterior jaw cartilages

by tough connective tissue, so that these three structures move as a unit on the posterior cartilages behind; and these latter, in turn, move on the quadrates.

At this stage the cartilages of the nasal capsule have begun to be laid down. The hind wall of the capsule is formed by a curved band of cartilage which stands up vertically from the inner end of the commissura along its front border (fig. 8, Pl. 36, *ppn.*). Behind, it abuts against the side wall of the neurocranium and fuses with it above and below, enclosing thus an orbitonasal foramen (*forn.*), through which nerve V_1 enters the nasal capsule o v e r the root of the commissura. At its upper end the band of cartilage is turned forward to make the roof (fig. 8, Pl. 36, *tn.*) of the nasal capsule and is continuous medially with cartilage (fig. 17, Pl. 39, *tn.*) which itself is continuous with the front, vertical wall of the neurocranium. At the side the roofing cartilage curves downward to form the lateral wall of the capsule behind. From the lateral wall, immediately above the front edge of the commissura, and resting on it, the rudiment of the 'processus antorbitalis' (fig. 8, Pl. 36, *pan.*, and fig. 17, Pl. 39, *pan.*) is seen to project straight forward. Its outer end, as also the free edges of the nasal roof, are, as yet, only formed in dense mesenchyme or in very young cartilage, but they have been drawn with firm outlines in the figures for the sake of clarity.

Farther forward in the nasal roof independent chondrification has begun (fig. 17, Pl. 39, *tn.*). From it the 'cartilago obliqua' (*co.*) projects backward and downward; the 'cartilago alaris' (*cal.*) is present and in front of it lies the 'cartilago prænasalis superior' (*cps.*); both these last in mesenchyme only. Also in mesenchyme, and lying on the upper surface of the trabecular horn, is the rudiment of the 'cartilago prænasalis inferior' (*cpi.*), while behind it the side wall and the lateral part of the floor of the nasal capsule are already present as young cartilage (*pn.*). A considerable nasal septum (*sn.*) is also developed in the middle line between the trabecular horns. This stage is only a little earlier than that figured obliquely from in front by Gaupp (1893, fig. 22).

A description of the neurocranium itself may be obtained

from Gaupp's accounts (1893 and 1906); but certain features closely affected by the changes in the jaws may be noted here. The optic foramen (fig. 1, Pl. 33, *fon.*), and the oculomotor foramen (*focn.*), lie immediately above the projecting ledge of the trabecula; this separates them from the two palatine foramina below for the carotid artery and its palatine branch—the 'foramen caroticum primarium' behind (*fcp.*), and the 'foramen cranio-palatinum' in front (*fc.*). The four foramina mark the upper and lower limits of the original trabecula, and their changes in size indicate the degree of destruction of this bar which takes place in later stages. As yet no destruction has taken place and the arrangement is typical of the tadpole skull.

Skulls Earlier than Stage 1.

Gaupp has figured and described such earlier skulls (his stage 2 and figs. 12 and 13). The skull figured as stage 1 in this paper has advanced towards the adult condition in that the rudiments of the antorbital process, and of the nasal cartilages, have developed. Earlier skulls differ in the absence of these cartilages. In a typical fully formed tadpole skull nerve V_1 passes over the root of the commissura, close against the side wall of the skull. Below it the anterior border of the commissura is thickened but as yet no antorbital process extends forwards from it, nor has the back wall of the nasal capsule grown up laterally to nerve V_1 or arched up over it to fuse above and below it with the cranial wall. V_1 is, therefore, not enclosed in an orbito-nasal foramen.

Apart from these differences the figures and description of stage 1 may be taken as typical of the tadpole skull.

Stage 2 (fig. 2, Pl. 33; fig. 9, Pl. 37; fig. 18, Pl. 40; fig. 20, Pl. 41).

External appearance: length of body, 11·7 mm.; length of tail, 20·5 mm.; hind legs more than 1 cm.; arms enclosed but bulging to the sides; body triangular; tail web narrowing; no horny jaw- or 'lip'-teeth present.

This stage shows a number of advances on stage 1. The lower jaw is set less transversely across the snout and this is particularly true of the posterior jaw cartilage (fig. 2, Pl. 33, *pjc.*, and fig. 18,

Pl. 40, *pjc.*); the anterior jaw cartilage (*ajc.*) is not curved back in the middle line. The posterior cartilage is undergoing a rotation on its long axis, so that its former dorsal surface is now facing d o r s o - l a t e r a l l y and its articular notch is facing backward and u p w a r d rather than backward and d o w n-w a r d . (These are significant differences in spite of the fact that animal 1 died with its mouth shut and animal 2 with its mouth open). Both jaw cartilages have been remodelled by a process of cartilage resorption; the matrix has been removed in places leaving only a rather indeterminate shell of peri-chondrium bounding now empty spaces. Only definitive carti-lage is shown in fig. 2, Pl. 33, but in fig. 18, Pl. 40, the former outlines of the pre-existing cartilage have been put in in dotted lines to assist description. A small area of cartilage matrix has been removed from the anterior lower face of the posterior jaw cartilage in front of the articular region, whilst a much greater erosion has taken place along its postero-ventral border; the external dotted line (fig. 18, Pl. 40) shows the posterior former limit, and the internal (anterior) dotted line the anterior limit of the matrix so removed. In the anterior jaw cartilage at least a third of its former volume of matrix has been removed along its antero-ventral face, whilst some erosion has also taken place posteriorly toward the middle line. The firm lines (fig. 18, Pl. 40) show the limits of the uneroded, definitive jaw cartilages.

The horny tooth blades have been lost from both jaws and the teeth have also been shed from the 'lips', which have become much reduced.

The three cartilage processes attaching the quadrate cartilage to the neurocranium have all undergone changes—either softening and distortion, or actual cartilage erosion. The cartilage along the posterior margin of the commissura has been removed, leaving only a ragged casing of perichondrium (dotted lines, fig. 9, Pl. 37, and fig. 18, Pl. 40); this erosion has completely destroyed the processus pseudopterygoideus. In addition, the destruction has extended to the subocular shelf along the side of the trabecula; it has taken place from the commissura to the ascending process. In front, the medial part of the trabecula remains as the ventro-lateral wall of the skull, but it shows a

raw outer edge from which the shelf has been eroded. Farther back, however, in the region of the optic and oculomotor foramina, the destruction has been more extensive, the whole thickness of the trabecula being involved. Below the optic foramen (fig. 2, Pl. 33, *fon.*) only the perichondrial walls of the trabecula remain, except for a ventral cartilage bridge just dorsal to the foramen cranio-palatinum (*fc.*). With the exception, therefore, of the presence of the perichondrium, the area of the optic foramen has been greatly increased ventrally at the expense of the trabecula. The presence of debris in this region is shown in fig. 2, Pl. 33, by a dotted margin to the former optic foramen; the foramen cranio-palatinum still retains uneroded margins.

The destruction has gone farther between the oculomotor foramen (*focn.*) and the foramen caroticum primarium (*fcp.*). The two foramina are now virtually joined into one, except for a bridge of perichondrium. Between the now enlarged optic and oculomotor foramina there still remains a narrow pillar of uneroded cartilage representing about half the original thickness of the trabecula. The destruction of the subocular shelf, the trabecula, the commissura, and the ascending process, and the softening of the top of the muscular process and of the tip of the tadpole otic process, all take place simultaneously. The changes are thus probably due to the same causes, which may well be some hormone release.

Now that the posterior angle between the commissura and the trabecula has been softened and freed of the obstruction offered by the subocular shelf and the processus pseudo-pterygoideus, the whole commissura has rotated backward about its attachment to the brain case, so that it slopes b a c k - w a r d s rather than forwards in passing out from the skull (compare fig. 8, Pl. 36, and fig. 9, Pl. 37). In addition, along its anterior edge, low down near the pars articularis, there has been some further slight erosion leading to a narrowing of width of the effective articulating surface of the quadrate (dotted line, fig. 9, Pl. 37).

The ascending process (*pa.*) has undergone destruction without any change in its position. The matrix of the middle of its

length has been removed and there remains only the vaguest trace of perichondrium joining its two ends. This residue of perichondrium is not shown in fig. 2, Pl. 33, and fig. 18, Pl. 40, which show only the stump projecting from the front border of the pro-otic foramen and the outer end projecting in from the quadrate cartilage (fig. 2, Pl. 33, *pa.*; fig. 9, Pl. 37, *pa.*; and fig. 18, Pl. 40, *pa.*).

The otic process (fig. 2, Pl. 33, *pot.*, and fig. 18, Pl. 40, *pot.*) has been softened, if one may judge by its buckled condition, and its subsequent partial destruction in stage 3. Its base, connecting it with the auditory capsule, has been extended, and the edge of the capsule itself has been widened laterally forming the beginning of a 'crista parotica' (fig. 9, Pl. 37, *cp.*). At its outer end the process has been forced backward and is now bent on itself (fig. 2, Pl. 33). Histologically its cells show no degeneration, and there is no loss of matrix.

The rotation about the top of the commissura has allowed the whole quadrate bar to move backward; it has apparently met with resistance from the otic process. As shown above, the otic process itself has been bent, but the outer edge of the quadrate cartilage has also been thrown into undulating folds (fig. 9, Pl. 37, and fig. 18, Pl. 40). There has been a resorption of matrix from the cartilage along its extreme lateral border in front of the otic process (dotted line, fig. 18, Pl. 40) but the perichondrium remains intact. In spite of the forces which must be acting in this process of buckling, the hind border of the quadrate— and its ascending process—have not moved back to any extent, and the lateral head vein and the hyomandibular branch of nerve VII lie uncrushed between them and the front face of the auditory capsule below the otic process.

In addition to moving backward the quadrate bar has become less horizontal and more vertical in position, thus opening up the angle which the whole bar makes with the cranial floor (compare fig. 8, Pl. 36, and fig. 9, Pl. 37). There is already a very slight tendency for the 'pars metapterygoidea' of the quadrate bar, in front of the notch (*nc.*) for the ceratohyal, to set more vertically than the remainder of the bar; this tendency is increased in subsequent stages.

The pars articularis (*paq.*) and the processus quadrato-ethmoidalis (*pqe.*) are, in this stage, still attached to the trabecular horn (fig. 2, Pl. 33, *ct.*) by the two ligaments shown in stage 1 (fig. 8, Pl. 36). The movement of the quadrate backward is therefore presumably responsible for the downward and backward bending of the trabecular horn (compare fig. 1, Pl. 33, and fig. 2, Pl. 33). The horn has undergone much degeneration particularly at its outer end where both its lateral and its median corners are reduced almost to shells of perichondrium; the former is much 'telescoped' on the more solid cartilage behind, but this telescoping is not shown in the figures.

Farther back the trabecular horn is being functionally replaced as the floor of the nasal capsule laterally by the growth of young cartilage on its upper face; whilst anteriorly its function as the skeleton of the snout is being taken over by the inferior pre-nasal cartilage (*cpi.*) which has increased greatly in size from the previous stage. Toward the middle line, however, the posterior part of the horn is still the floor of the nasal capsule; its inner edge has become fused with a lateral outgrowth from the base of the nasal septum (*sn.*)

The roof of the nasal capsule has become complete by the fusion of the various roof elements with one another and with the top of the nasal septum. These changes, coupled with the downward deflection of the trabecular horn and of the nasal capsule above it, have greatly altered the appearance of the dorsal view (fig. 18, Pl. 40) from that of stage 1 (fig. 17, Pl. 39). The other complicated nasal structures will not be dealt with here, but attention must be directed to the antorbital process (*pan.*). It has developed considerably from the previous stage so that now its outer end has become a flattened plate bifurcated laterally. The forwardly directed 'processus maxillaris anterior' (fig. 2, Pl. 33, *pma.*, and fig. 9, Pl. 37, *pma.*) is the first to be developed and is already present, in miniature, as the tip of the process in stage 1 (fig. 8, Pl. 36). The 'processus maxillaris posterior' *pmp.*), on the other hand, is still incompletely chondrified. Its inner end curves backwards and inwards, and is laid against the tip of the processus quadrato-ethmoidalis

(*pqe.*) of the quadrate, on its outer side. The inner end of the process is still formed in mesenchyme only, whilst more laterally the tissue becomes denser and passes into the young cartilage which comprises the whole outer end of the antorbital process.

The base of the antorbital process—the 'pars plana' (*ppn.*) rests on the front border of the commissura from which it can be distinguished histologically by the difference in the amount of matrix in its cartilage. The rotation of the commissura mentioned above leads necessarily to the rotation of the antorbital process above it. Consequently the axis of this process, which in stage 1 pointed forward, now points forward, downward, and outward.

Like the trabecular horn, the supra-rostral cartilage has undergone very considerable reduction. Except for its lateral wings the whole of its cartilage is showing a reduction of its matrix, and the narrow ventral bridge in the middle line has already broken through (fig. 18, Pl. 40, *cls.*), thus dividing it into lateral halves. Emargination has taken place at its upper and lower edges so that its dorso-ventral depth has been greatly reduced (compare figs. 1 and 3, Pl. 33). Like the lower jaw, it has lost the horny tooth blade which underlies it in the typical tadpole.

Many of the changes so far mentioned are related to the backward movement of the quadrate, but the changes in the muscular process cannot be attributed to this cause. The upper edge of the muscular process (fig. 2, Pl. 33, *pmq.*) has become softened and some matrix absorption has taken place. The orbito hyoideus muscle takes its origin all round the edge of the process and is inserted on the ceratohyal (fig. 2, Pl. 33, *ch.*) below. The pull of this muscle is apparently responsible for the downfolding of the upper edge of the process which has become turned over, outwards, and downwards (see fig. 2, Pl. 33, and fig. 18, Pl. 40), thus reducing the height of the process and allowing the attachment of the muscle to pass more ventrally. [This procedure is not always followed; in some animals the upper edge of the process folds down on itself like the folds of a closed fan, and is not turned outwards. However, in all

cases the height is reduced, the peripheral cartilage being first softened and then destroyed.]

For clarity the muscular process is drawn with firm lines in fig. 2, Pl. 33, and fig. 18, Pl. 40.

There remains for description one other structure related to the jaws which makes its appearance at this stage for the first time. This is the rudiment of the 'processus basalis (quadrati!)' of Gaupp (fig. 20, Pl. 41). To avoid the implications of this name it will be called the 'pseudobasal process' throughout this paper, and its probable homology will be suggested in the discussion which follows. [The name 'pseudobasal articulation' was first used by de Beer, 1926, p. 336, for the articulation of this process with the auditory capsule in the adult frog. Following Gaupp and other workers, de Beer assumed the process to be of quadrate origin—a view with which the present author disagrees—but his nomenclature may usefully be retained in this connexion.]

The subocular palatal vacuity of the tadpole skull is floored by a sheet of fibrous connective tissue which is slung from the bounding structures of the vacuity, i.e. from the inner and outer edges of the quadrate bar laterally, the hind edge of the commissura and the processus pseudo-pterygoideus in front, the subocular trabecular shelf medially, the lower edge of the prootic foramen, and the face of the auditory capsule behind. On the auditory capsule the sheet is attached somewhat ventrally to the ascending process and, laterally, swings upwards toward the front edge of the fenestra ovalis. It is in this latter region that pseudobasal process begins to condense (fig. 20, Pl. 41). In stage 1 the subocular sheet is already somewhat more fibrous in this region, while in stage 2 there has been a very considerable increase in the number of cells here, so that there is a patch of denser mesenchyme, with fairly sharp limits, lying in front of the ventro-lateral, anterior face of the auditory capsule. At its postero-lateral corner the cells of this mesenchyme condensation appear to have migrated out from the front face of the capsule whose cartilage in this region is histologically young, for there is no perichondrium over it and the scatter of nuclei is continuous from the capsule to the pseudobasal process.

More medially, however, the perichondrium of the capsule is intact, so that the pseudobasal process, in this region, is probably a condensation in situ. In front the process passes into the subocular sheet and medially into the long fibrous strands which attach the fibres of the pterygoid muscle to the front, lower face of the auditory capsule. The limits of the process are sharp enough to allow of its reconstruction, and fig. 20, Pl. 41, shows it as a conical structure with a concave upper surface which underlies the back of the quadrate bar. The perichondrium of the quadrate in this region is intact and there is no indication whatsoever that the pseudobasal process owes its origin in any way to the quadrate, as Gaupp believed. The broad base of the cone is applied to the auditory capsule, the more intimate connexion being shown laterally and behind. The hyomandibular branch of nerve VII and the lateral head vein run out laterally along its upper surface close in front of the auditory capsule; VII palatine leaves the pro-otic foramen medially (morphologically anteriorly) to it, whilst the carotid artery passes from its lateral position behind, to its medial position in front, below the process.

Stage 3 (fig. 3, Pl. 34; fig. 10, Pl. 37; and fig. 19, Pl. 41).

External appearance: length of body, 9 mm.; length of tail, 18 mm.; hind legs large and flexed at the knee; arms free; fin web of tail very narrow; corners of mouth almost back to the eye.

[This animal has an unusually small skull, and is also peculiar in having no trace of the cartilage roof of the neurocranium other than the tectum synoticum.]

The changes mentioned in the previous stage have been continued and accentuated in this stage.

The two elements of the lower jaw are coming more into line with one another (fig. 3, Pl. 34), and they are more closely pressed together; they are beginning to fuse at the joint, though the division is still clearly marked externally, and internally is shown by the enclosure of perichondrium. There has been a further rotation of the posterior jaw cartilage on its long axis

so that now the articular notch (fig. 19, Pl. 41, *an.*) faces directly upward and the anterior spur, which in stage 2 o v e r h u n g the anterior element, now lies along its o u t e r, side and embraces it (fig. 3, Pl. 34). What is now the most anterior upper point of the posterior cartilage is being eroded away (compare fig. 18, Pl. 40, and fig. 19, Pl. 41). The anterior jaw element has become hooked backwards at its outer end, whilst its inner end has been trimmed by erosion into a small rounded rod with a clubbed inner end which is somewhat upturned in the middle line. From the histology of the cartilage, as well as by a comparison of fig. 18, Pl. 40, and fig. 19, Pl. 41, it may be seen that both jaw elements are growing in length; this is particularly true of the posterior element, when it is remembered that the animal of stage 3 is so much smaller than that of stage 2. The result of the straightening and growth of the jaw is shown in the more posterior position now taken up by its articular region (compare fig. 2, Pl. 33, with fig. 3, Pl. 34).

In this stage much of the commissura has been entirely destroyed; the destruction has taken place in the angle between it and the skull wall, and has progressed from behind forwards. The antorbital process in consequence is now becoming free of the commissura whose cartilage only remains as a ragged lower posterior edge to it (dotted line, fig. 19, Pl. 41); more laterally, however, the connexion still remains intact, though the matrix of the commissura is even here much eroded. Laterally to this again, the cartilage of the former commissura, far from being eroded, is showing an increase in the number of its nuclei—an indication of active life. This active cartilage (fig. 3, Pl. 34, *ptc.*; fig. 10, Pl. 37, *ptc.*; and fig. 19, Pl. 41, *ptc.*) is that part of the commissural band from which the adult pterygoid process will be carved out, by the erosion of the neighbouring cartilage from its surface, both above and below. In front, this pterygoid bar (*ptc.*) is continued into the projecting processus quadrato-ethmoidalis (*pqe.*) and, with it, is destined to give the whole adult pterygoid process. [The two names of the parts of what becomes a single adult structure are retained for convenience of description.] What remains then of the commissura at this stage is the adult pterygoid process and a band of cartilage

debris on its upper and inner side still connecting it to the antorbital process. Cartilage is also being destroyed on the under side of the future pterygoid process, between it and the pars articularis of the quadrate (dotted line, fig. 10, Pl. 37). This is allowing the process to emerge as a round rod projecting from the inner edge of the quadrate cartilage. At the same time it is narrowing the width of the quadrate bar; a similar erosion of cartilage below the muscular process, laterally, is assisting in this narrowing process (dotted line, fig. 19, Pl. 41).

This animal has not advanced so far as that of stage 2 in the destruction of the trabecula. The subocular ledge has, of course, been destroyed and so has some of the outer cartilage of the trabecula, between the commissura and the front border of the pro-otic foramen, but there yet remains much solid matrix separating the foramina—matrix which in stage 2 had already been destroyed.

The angle which the whole quadrate bar makes with the cranial floor, as seen in side view, has been increased by about 5° and the whole bar has been moved farther backward (compare fig. 2, Pl. 33, with fig. 3, Pl. 34). The folds at the back of the quadrate, seen in fig. 9, Pl. 37, have been compressed (fig. 10, Pl. 37), and the matrix of the cartilage so affected is being rapidly dissolved away, especially laterally, leaving only a casing of perichondrium. This is true also of the anterior end of the otic process which now only makes a perichondrial connexion with the edge of the quadrate bar. Farther back the otic process is quite uneroded, and, behind it, is a slightly thickened crista parotica. [In another specimen of this stage I have found the otic connexion entirely destroyed, the crista absent, and the folds of the quadrate bar already much more fully destroyed; but even in this tadpole, which is an exception in this, the back of the quadrate does not touch the front face of the auditory capsule.] So, too, here (stage 3) the only contact between the quadrate and the auditory capsule is through the perichondrium of the otic process.

The folds at the back of the quadrate are rising up above the level of the remainder of the bar, which, between them and the notch for the ceratohyal (fig. 10, Pl. 37, *nc.*) is being depressed

in a gentle curve. In front of the otic process the outer edge of the bar is represented only by perichondrium which is much twisted and buckled (fig. 3, Pl. 34). It continues forward into the out-turned upper edge of the muscular process (*pmq.*), whose height has been further reduced (compare fig. 2, Pl. 33, with fig. 3, Pl. 34). Medially, the whole inner border of the quadrate is being eroded from the commissura back to the auditory capsule. This destruction has entirely removed the stump of the ascending process from the quadrate; the inner end of this process has also been removed from the front border of the pro-otic foramen. So that all trace of the ascending process has been lost between stages 1 and 3; it contributes nothing to the adult skull in Rana.

The matrix in the centre of the quadrate bar is being removed in the region of the notch for the ceratohyal; here the cartilage was already thin (figs. 9 and 10, Pl. 37, *nc.*), and was narrow laterally. As a result, this is a point of weakness in the bar and, already in this stage (fig. 10, Pl. 37), we see the second main process of buckling beginning. This consists in the bending of the bar about the notch, so that the part behind becomes depressed into a U, as mentioned above, while the part in front bends downward and backward to become more vertical. The whole change is seen in a more advanced state in stage 4 (fig. 11, Pl. 37), and it is completed in stage 5 (fig. 12, Pl. 37).

Before leaving the quadrate, mention must be made of a patch of young cartilage lying close-pressed against the under side of this bar, just behind and above the pars articularis (fig. 10, Pl. 37, *psq*). As yet it is still marked off from the quadrate by the perichondrium of the latter, but it will later fuse with it completely, and will grow into the spur which projects backwards and increases the articular surface. (In this animal there is also some young cartilage opposite it, on the front face of the quadrate; this, however, seems to be exceptional and is not found in any other specimens of this and other stages; it is not figured as it occurs laterally to the cut in fig. 10, Pl. 37.)

A reconstruction of the pseudobasal process (not figured) shows it to be just as in stage 2. It is still only formed in quite sparse mesenchyme, with the exception of the outer part. In

this animal this lateral part happens to be already chondrified as a cap of cartilage on the face of the auditory capsule (fig. 3, Pl. 34). No perichondrium intervenes between them laterally, whilst, in front, its cells pass insensibly into the mesenchyme of the remainder of the process. The early chondrification in this region is probably exceptional.

The supra-rostral cartilage has been entirely destroyed and so has the outer half of the trabecular horn; only a little, faintly marked, debris in the upper 'lip' remains. The root of the trabecular horn, however, is still present as the floor of the nasal capsule; at present it ends raggedly in front (dotted line, fig. 3, Pl. 34, ct.), at the level of the outer end of the nasal septum. The young cartilage from the floor of the nasal capsule in front, and from the root of the antorbital process behind has grown in and has joined medially to the internal nostril along the lateral border of the horn. Now that the horn has been so much reduced the two ligaments shown in fig. 8, Pl. 34, have become detached from it anteriorly; but they still are easily detectable behind.

The antorbital process has increased in size. Its base has extended inwards considerably, at the expense of the reduced commissura, and is widely inserted on the antero-lateral face of the end of the neurocranium, below nerve V_1, in the position formerly occupied by the commissural attachment. Its free end is now longer, and therefore projects farther laterally than in stage 2. Its processus maxillaris posterior has increased in size and length and is now fully chondrified; its inner end is fused against the outer side of the processus quadrato-ethmoidalis which has been turned outward to meet it.

S t a g e 4 (fig. 4, Pl. 34; fig. 11, Pl. 37; fig. 13, Pl. 38; fig. 21, Pl. 42).

E x t e r n a l   a p p e a r a n c e: length of body, 9 mm.; length of tail, 13 mm.; very little fin web; all limbs free and large; corners of mouth under the eye; frog-like appearance except for tail.

The changes between stages 3 and 4 are not very great. The lower jaw shows a slight increase in length, especially in the

anterior jaw cartilage; but much of the difference in size be-
tween the jaws of fig. 19, Pl. 41, and fig. 21, Pl. 42, can be
accounted for by the different absolute sizes of the skulls of
the animals concerned. The jaw as a whole has moved back
so that its anterior end protrudes less far forward into the snout,
while its articular region is now coming under the optic foramen.
The two elements are far more closely fused and very little
trace of the double origin of the jaw is visible externally; even
internally the enclosed perichondrium is beginning to disappear.
Throughout the length of the jaw the cartilage shows an in-
creased number of nuclei and gives a histological appearance
of active growth. All erosion of cartilage has now ceased.

What remains of the commissura has been detached from the
base of the antorbital process and lies as an eroded plate over-
lying the pterygoid process, along its upper and inner side (fig.
21, Pl. 42, *cqa.*; fig. 4, Pl. 34, *cqa.*; fig. 11, Pl. 37, *cqa.*; fig. 13, Pl. 38,
cqa.). The quadrate bar has moved farther back and its forward
extremity is becoming more erect (fig. 11, Pl. 37). As a result
of this rotation the pterygoid process (*ptc.*) is coming to lie
more horizontally. It should be noted also that, whereas in
stage 1 the quadrate bar was so inclined that it faced inwards,
now, in stage 4, it faces almost directly forward, without the
inward tilt. This tendency, begun in stage 3, is possibly due
to the backward pressure exerted by the jaw on the outer part
of the pars articularis at a time when the inner border is still
anchored by the commissura and the processus maxillaris
posterior. In any case it results in the anterior end of the
pterygoid process passing more and more laterally away from
the skull wall. In fig. 21, Pl. 42, it is seen now to point inwards
only to a slight extent compared with fig. 19, Pl. 41; the break-
down of the commissural attachment to the antorbital process
must assist this movement. The change of tilt of the quadrate
is obscured by the apparent replacement, in the figures, of the
lowest corner of the quadrate (fig. 1, Pl. 33 and fig. 8, Pl. 36) by the
posterior spur (fig. 4, Pl. 34, and fig. 11, Pl. 37), but it is notice-
able in that the muscular process is caused to stand up more
vertically and to lean less toward the middle line (compare
fig. 17, Pl. 39, and fig. 19, Pl. 41).

The outer edge of the quadrate bar is more buckled and is formed only of perichondrium. Behind it the root of the otic process is thickened into a strong knob; the outer end of the otic process is bent back and has been carried underneath this knob. A cloud of mesenchyme surrounds these structures laterally, and stretches forward toward the muscular process, lying along the outer side of the quadrate debris; it will subsequently chondrify as the adult crista parotica.

The small folds of cartilage at the posterior end of the quadrate (fig. 11, Pl. 37) are less eroded in this animal than in stage 3; they are still parted by a space from the face of the auditory capsule. Between them and the notch (*nc.*) for the ceratohyal the main quadrate bar is folding into a U (fig. 11, Pl. 37). The inner under side of this U rests on the concave upper face of the pseudobasal process (fig. 13, Pl. 38, *psp.*) and does much to mould it. The process, in its turn, probably prevents the quadrate from collapsing back against the auditory capsule and it also forces the whole bar upward against the under side of the otic process. The pseudobasal process is now made up of dense mesenchyme, but no chondrification has yet begun, even laterally. The lateral head vein has been displaced to a position largely above the folded quadrate whilst nerve VII hyomandibular passes through the narrow space just above the pseudobasal process behind the quadrate.

The antorbital process shows some increase in size and also a change of position. It now stands out more laterally from the skull (compare fig. 19, Pl. 41, and fig. 21, Pl. 42). The change is due to an actual backward rotation, which is made possible by the softening of the cartilage between its root and the skull wall; this cartilage is the last remnant of the commissural root and is now being removed and replaced by the younger cartilage of the antorbital process, which is usurping its base of attachment. The rotation has allowed the whole palato-pterygoid bar to move back without any marked increase in its length; it has also increased the distance between the front border of the antorbital process and the nasal cartilages (compare figs. 3 and 4, Pl. 34). The under edge of the process still shows a

ragged scar where the commissura has been detached (fig. 21, Pl. 42, *cqa.*, and dotted line).

The nasal cartilages have attained their adult form with the root of the trabecular horn incorporated as the floor of the capsule; the ragged outer end of the horn has been smoothed out by partial erosion. In this animal, too, the adult arrangement of the foramina in the orbital region has almost been attained. That is to say, the optic foramen has greatly increased its area at the expense of the trabecula below it; it is only separated off from the foramen cranio-palatinum (fig. 21, Pl. 42, *fc.*, and fig. 4, Pl. 34, *fc.*) by a narrow cartilage bridge; this bridge is lost in adult animals (fig. 6, Pl. 35, and fig. 7, Pl. 36). The oculomotor foramen and the foramen caroticum primarium are joined into a single large opening (fig. 21, Pl. 42, *focn.*, and fig. 4, Pl. 34, *focn.*) thereby obliterating all trace of the trabecula in this region. [In the Cystignathid frog, C r i n i a G e o r g i a n a, du Toit (1934) finds that these two foramina remain separate even in the adult.]

S t a g e 5 (fig. 5, Pl. 35; fig. 12, Pl. 37; fig. 14, Pl. 38; and figs. 22 and 23, Pl. 43.)

E x t e r n a l a p p e a r a n c e: length of body, 13 mm.; length of tail stump, 2 mm.; corners of mouth reaching back to the hind border of the eye.

In the 36 hours which have elapsed since stage 4, the changes in the jaws have been far-reaching. Once the commissura is detached from the antorbital process, the buckling of the quadrate takes place rather quickly. The whole quadrate has moved backward and has risen upward so that the top of the muscular process is on a level with the otic process. At the same time its front end—the 'pars metapterygoidea'—has become more erect by the backward rotation of its lower end (fig. 5, Pl. 35, and fig. 12, Pl. 37). Behind what may now be seen to be the definitive adult quadrate bar, the remainder of the quadrate has been folded on itself by the closing of the U mentioned above. The notch for the ceratohyal has closed on itself (fig. 5, Pl. 35, *nc.*, and fig. 12, Pl. 37, *nc.*). The upper posterior end of the

original quadrate bar stands up above the definitive quadrate, between it and the auditory capsule (fig. 14, Pl. 38, *qd.*); it is now little more than a hollow shell of perichondrium, whose folding still shows the arrangement of small undulations of earlier stages (fig. 12, Pl. 37, *qd.*); it is beginning to fall forward and cover the upper end of the adult quadrate bar.

The lateral edge of the auditory capsule is now somewhat expanded, by the growth of its own cartilage cells, into a projecting ledge—the crista parotica (fig. 5, Pl. 35, *cp.*). The perichondrial covering of this ledge is lost and its cells are continuous with those of a surrounding mass of dense mesenchyme, the inner part of which is already chondrified as very young cartilage continuous with the older, histologically distinguishable, cartilage of the auditory capsule. This mesenchyme passes forwards half the way along the upper edge of the muscular process and, medially, has grown into the spaces in the debris of the degenerate quadrate bar (the full complication of these debris is not shown in fig. 5, Pl. 35). The mesenchyme lies laterally to the muscular process, but median to the top of the squamosal which is now growing upward to cover this region.

For the first time in this series of stages the debris of the quadrate have collapsed backward so that they are in contact with the front face of the auditory capsule (fig. 12, Pl. 37). As a consequence, the lumen of the lateral head vein has been almost closed over part of its length and nerve VII hyomandibular is squashed between the two structures. What remains of the tip of the (tadpole) otic process has been twisted inwards below the crista against whose front face it is impacted along with the debris of the quadrate. There is, in consequence, a buckled mass of degenerate cartilage and perichondrium compressed between the muscular process in front and the crista and the auditory capsule behind. Intermingled with it are cells which have grown inwards from the lateral band of mesenchyme mentioned above.

The collapse of the quadrate has had its effect also on the pseudobasal process below and behind it (fig. 14, Pl. 38, *psp.*, and fig. 23, Pl. 43, *psp.*). Where the pressure was greatest the soft tissue of this process has been forced to the two sides with

the result that its upper border has been notched (dotted line, fig. 14, Pl. 38, and fig. 23, Pl. 43). Toward the middle line the inner upper edge of the definitive quadrate bar (just above the root of the pterygoid process) has also cut into its front edge, again notching it (fig. 23, Pl. 43, *nq.*). Here the quadrate and the pseudobasal process come into very intimate contact, for the quadrate in this region is already devoid of perichondrium (fig. 12, Pl. 42, dotted line). More medially still, the two structures are parted by the inclusion of a shelf of the developing pterygoid bone in the joint. As yet the pseudobasal process is unchondrified.

The shaping of the quadrate bar and the reduction of its width above the pars articularis is still going on; this process was mentioned on p. 503 in stage 3. Laterally, a curved notch is being removed, by matrix absorption, between the pars articularis and the muscular process and similar erosion is taking place below the pterygoid process on its inner side (compare widths, figs. 14 and 13, Pl. 38).

All trace of the commissura has been removed from the upper surface of the pterygoid bar, which has now been shaped into a round rod (fig. 14, Pl. 38, *ptc.*). Its anterior end—the processus quadrato ethmoidalis—is still inclined at a slight angle to that part of the bar which was cut out of the commissura (fig. 12, Pl. 37, *pqe.* and *ptc.*), but this angle is already much smaller than in stage 4 (compare figs. 12 and 11, Pl. 37). The whole bar lies horizontally (fig. 5, Pl. 35), but still slopes slightly inwards at its anterior end (fig. 22, Pl. 43); it makes an angle with the quadrate bar which is still less than a right angle below (fig. 5, Pl. 35).

The antorbital process has again rotated farther backward (compare fig. 21, Pl. 42, and fig. 22, Pl. 43). The movement, coupled with a slight increase in the length of its processus maxillaris posterior, has allowed the quadrate to take up its more posterior position. The whole palato-pterygoid bar, formed as it is of the processus maxillaris posterior in front, the processus quadrato ethmoidalis medially, and the (commissural) pterygoid process behind, has become much straightened and now lies approximately parallel to the cranial floor. The last

debris of the commissura have been removed from the back of the antorbital process.

There is a considerable difference in the absolute sizes of the animals of stages 4 and 5; when this has been taken into account it will be seen that there has been an increase in the length of the lower jaw; this applies to both jaw cartilages though more particularly to the posterior element. As a result the articular region has passed behind the level of the optic foramen, and the anterior end has again been slightly withdrawn from the snout. This latter change, however, may rather be due to the downward and backward flexure of the jaw in the symphysial region. The apparent distortion in this region has been noted also in victoria blue preparations of similar stages of development. The general set of the jaw has altered; it now lies parallel to the cranial floor and to the upper jaw, instead of sloping upward in front. Though invisible now from the outside, the point of junction of the jaw elements may still be found in sections; it has been put in in dotted lines in fig. 5, Pl. 35, and fig. 22, Pl. 43, for convenience of comparison with the other figures.

Stage 6 (fig. 6, Pl. 35; fig. 15, Pl. 38; fig. 24, Pl. 43).

External appearance: length of body, 11·8 mm.; minute tail stump; corner of mouth behind the eye.

The lower jaw now shows a great increase in length and at the same time a decrease in area of transverse section. As there has been no active process of erosion taking place, it is to be assumed that the growth in length may be due rather to a stretching or redistribution of material than to an addition of new material. This elongation—whatever its method—is restricted entirely to the posterior jaw cartilage. The downward and backward flexure of the anterior end of the jaw has been reduced to more normal proportions.

The antorbital process has rotated a little further, particularly at its outer end, and the upper jaw cartilages have become somewhat elongated and are much straightened. The increase in length seems to have taken place equally in the processus maxillaris posterior and in the pterygoid process; this is the

first marked growth of this latter process, in the series here described. In dorsal view (not figured) the pterygoid process is seen to curve outwards at its anterior end on passing forward from the quadrate; this is characteristic of the adult skull (fig. 25, Pl. 44) and had not previously been attained in the series.

The quadrate bar has rotated backward at its lower end so that the angle which it makes with the pterygoid process above has become obtuse; in stage 5 it was still acute. As a result of the rotation the muscular process slopes downwards and forwards from the crista parotica with which it is now in cartilagenous continuity. The width of the quadrate bar appears to have been further reduced, but all erosion has now ceased (compare figs. 14 and 15, Pl. 38). Behind the pars articularis the posterior spur of the quadrate has increased in size (fig. 6, Pl. 35, *psq.*).

Special attention must now be given to the manner in which the adult quadrate bar is attached to the neurocranium. It is a difficult problem and its elucidation from the study of sections is rendered difficult by the large amount of degenerate cartilage and debris in this region, as well as by the dense mesenchyme laterally. However, the present method of reconstruction has been very helpful, and the pictures so obtained give a clear answer to the problem. A close comparison of figs. 13, 14, 15, and 16, Pl. 38, makes the position clear.

The otic process of the adult, so far as this is supplied by the quadrate, is formed of what remains of the base of the muscular process of the tadpole; that is, its anterior basal part which lies in front of the notch for the ceratohyal. This is clearly seen in the series of lateral views of the skull. The remainder of the adult quadrate bar—its 'pars metapterygoidea'—is made up of that part of the tadpole quadrate which lies in front of this notch, i.e. in front of a line from the middle of the muscular process, on its inner side, to the back of the pterygoid process. This line is seen as the sloping upper end of the quadrate in fig. 14, Pl. 38, as a darkly shaded line in fig. 13, Pl. 38, and as a dot and dash line in fig. 15, Pl. 38 (–·–·–·–). The folded remainder of the quadrate bar lies behind the pars metapterygoidea in

fig. 14, Pl. 38, and rises up above it. Laterally lie the debris of the back of the muscular process, of the buckled lateral edge of the quadrate, and of the tip of the tadpole otic process (fig. 5, Pl. 35). The soft, partly chondrified pseudobasal process lies on the inner side of the adult bar (fig. 14, Pl. 38, *psp.*).

In the time which elapses between stages 5 and 6, most of the matrix has been removed from the buckled posterior part of the quadrate (fig. 12, Pl. 39, *qd.*), thus reducing its bulk and leaving chiefly the more resistant perichondrium. The shell so formed then falls, or is forced, forward and folds itself over the upper edge of the definitive quadrate bar like a ridge-tile (compare figs. 14 and 15, Pl. 38, *qd.*). At the same time the fold becomes fused on its inner side to the pseudobasal process. The quadrate cartilage has already cut into the soft tissue of this pseudobasal process, making a groove (fig. 23, Pl. 43, *nq.*) whose upper part also lies over the top of the quadrate bar as a ridge-tile. Though in very intimate contact with this overlying fold of dual origin the quadrate bar can still be clearly distinguished from it histologically; in fig. 24, Pl. 43, therefore, the quadrate bar has been removed and the pseudobasal process, *psp.*, is shown with the quadrate element, *qd.*, joined to its antero-lateral face; behind this quadrate tissue is the groove, *nq.*, in which lies the definitive quadrate bar. In fig. 15, Pl. 38, the posterior limit of the pseudobasal process is shown by a dotted line (. . . . .) and the lateral limit of the auditory capsule (with the root of the tadpole otic process) by a broken line (- - - - -).

The attachment of the muscular process (= adult otic process of the quadrate) to the auditory capsule is also of dual origin. (1) The debris (of the muscular process, of the lateral edge of the quadrate, and of the tip of the tadpole otic process), with a probable infilling of mesenchyme cells from the lateral source, are by some means converted into a mass of cartilage; it lies between the auditory capsule with its tadpole otic process behind, and the back of the muscular process (adult otic process) in front. Its appearance in sections is histologically a little different from cartilage formed directly from mesenchyme, and furthermore it contains liberal streaks of perichondrium within

its substance, streaks which still show the original folding of the quadrate in this region. (2) On passing laterally this peculiar cartilage shades off imperceptibly into more typical young cartilage formed by direct condensation from the lateral band of mesenchyme mentioned above. This young cartilage lies along the outer upper edge of the muscular process (adult otic process), almost to its anterior border; it is now covered by the squamosal which has grown up laterally to it. Behind, it forms a lateral casing round the tadpole otic process and the original tadpole crista parotica of the auditory capsule, and extends to the side and curves downward as an overhanging roof to the region of the middle ear. Together all these structures form the adult crista parotica (fig. 6, Pl. 35, *cp.*) and the otic attachment (*poa.*).

The pseudobasal process is chondrified, for the first time in the series, as young cartilage. Between its base and the auditory capsule there now lies a thin sheet of dense fibrous tissue making a joint between them. Similarly, the root of the pterygoid process is separated from the pseudobasal process by fibrous tissue which is becoming calcified as part of the pterygoid bone; dorso-laterally, however, the two structures are fused together.

Too much attention cannot be given to these quadrate attachments in view of the interpretation put on them by recent workers (Kruijtzer, 1931). It may be pointed out, then, that the definitive quadrate bar may be separated from the neurocranium, and from its temporal attachments, in virtue of the different histological character of its older cartilage. When this has been done, a bridge of cartilage is left which arches over the cranio-quadrate passage for nerve VII hyomandibular and the lateral head vein. This bridge is made up of three main elements; (1) the pseudobasal process medially; (2a) the middle part of the back of the folded quadrate bar of the tadpole, i.e. the 'ridge-tile' mentioned above; (2b) the lateral debris of the quadrate bar, its muscular and (tadpole) otic processes, all of which are reconverted into true cartilage; and (3) the band of lateral mesenchyme cartilage fused on laterally. It cannot be too fully stressed that the middle of this bridge, in R a n a, is composed of c o n v e r t e d q u a d r a t e t i s s u e.

Stage 7 (fig. 7, Pl. 36; fig. 16, Pl. 38; fig. 25, Pl. 44, and fig. 31, Pl. 45).

External appearance: typical young frog some weeks after leaving the water; it had fed on land; no tail; body length, 14·3 mm.

The animal had begun to grow again after the metamorphosis. Its skull is the first in the series to show a general increase in size in all its structures. (During metamorphosis there seems to be a tendency for the skull to d e c r e a s e slightly in size.)

The lower jaw again shows a considerable increase in length and, as in the previous stage, this growth is restricted to the posterior jaw cartilage almost entirely (compare fig. 6, Pl. 35, and fig. 7, Pl. 36). The diameter of its cross-section is slightly reduced, suggesting a farther stretching of its material. As a result of the growth the articular region has passed yet farther back.

To accommodate the now elongated jaw the quadrate has rotated backward about its upper end, thereby changing the slope both of the muscular process and of the crista parotica behind (compare fig. 6, Pl. 35, and fig. 7, Pl. 36). As a result, too, the pterygoid process has undergone a slight downward rotation, though to a smaller extent than the quadrate bar, so that the angle between them has again become more obtuse. The pterygoid process has also rotated outward at its anterior end (fig. 16, Pl. 38, *ptc.*, and fig. 25, Pl. 44, *ptc.*).

The various elements of the otic and pseudobasal processes have become more fully fused and are therefore the less easily distinguishable. The amount of cartilage in this region has been reduced, especially laterally, where the otic process and the upper end of the quadrate bar are of more delicate build. The otic attachment, however, still shows flecks of perichondrium in its substance; whilst the cap of cartilage may still be traced running down from it, along the top of the quadrate bar to the pseudobasal process. But these distinctions are now purely histological and are no longer structural. It is to be noticed that it is only the outer, upper side of the pseudobasal process which is fused with the quadrate (fig. 16, Pl. 38); medially the pseudobasal process (marked with a dotted line (....) is separated

by a cleft (which contains part of the pterygoid bone) from the root of the pterygoid process. At the back of the pars articularis the spur (fig. 7, Pl. 36, *psq.*) has become much enlarged and now takes part in the actual articulating surface.

For the rest, this stage differs little from the previous stage or from the typical adult frog's chondrocranium. The antorbital process slopes backward to a more marked degree and its outer end has taken up a more ventral position, thereby carrying the processus maxillaris posterior downward with it. In dorsal view the whole upper jaw is seen to bow out to the side, whilst its two elements have now come fully into line with one another (compare fig. 22, Pl. 43, and fig. 25, Pl. 44).

The Skull of Frogs older than Stage 7.

The changes noted in older skulls are continuations of the changes begun in earlier stages.

The quadrate bar rotates yet farther backward. The middle of its length is firmly fused to the pseudobasal process and its upper end to the crista parotica; consequently it is only its lower end which is free to move to any great extent. Its backward movement, therefore, bends the bar on the pseudobasal process as a fulcrum. The lower end of the bar lies close under the auditory capsule and is only parted from it by the width of the hyoid cornu which intervenes between them. The appearance of increased backward rotation is enhanced by the increase in the size of the posterior spur, which has grown upward and backward from the now almost horizontal pars articularis. The horizontal position of this region, coupled with the increase of the spur, supplies the quadrate with a much greater articulating surface for the lower jaw. This is met by an increase in the size of the articular region of the jaw, which has become extended into a clubbed end with a notch in the upper surface to fit on the quadrate; in front of the articular region the transverse section of the jaw is relatively smaller than in earlier stages.

But beside rotating backward the quadrate bar has undergone another change of position. In stage 7 its flat anterior face was directed f o r w a r d and downwards, but in later stages it comes to face o u t w a r d as well as forward and downward.

This applies chiefly to the region lateral to the pterygoid process, where the outer edge has been twisted backward, while the median edge has been held in its former position by the buttressing action of the pseudobasal process behind it. More ventrally the bar again becomes partially untwisted so that it once more faces forward in the articular region. This lateral twisting is but the completion of a process already beginning in stage 2; it is responsible, in part, for the outward rotation of the pterygoid process.

The otic attachment of the quadrate does not seem to have been entirely rigid in stage 7, since the postero-lateral corner of the crista parotica has come to be twisted upward above the neighbouring wall of the auditory capsule. This is probably due to the rotation of the quadrate and the consequent change in slope of the muscular process and therefore of the crista behind it. Further, the crista shows a change, in that part of its lateral border is now continuous with part of the upper border of the annulus tympanicus; while on its lower surface there is a fusion with the extra-stapedial of the middle ear apparatus; this last had already taken place in stage 7.

This description is taken from my own reconstructions of the temporal region of young adult frogs—both in anterior and lateral views. It was supplemented by a study of a solid reconstruction of this region made by Dr. J. W. Jenkinson and now in the University Museum, Oxford.

(a) Short Summary of the Metamorphosis.

The lower jaw cartilages increase in length and decrease in thickness; part of the decrease is due to erosion and part, possibly, to a stretching process. The anterior cartilage elongates slightly at first, but very little later; it becomes more horizontal and its outer posterior end hooks backwards and begins to fuse with the posterior cartilage. This latter cartilage rotates on its long axis so that its former upper surface now lies laterally and its upstanding innermost peak lies laterally beside the anterior cartilage, with which it fuses. Its S shape is straightened out largely by erosion and it then increases in length and comes to lie with its long axis more nearly parallel

to that of the body, instead of transversely to it. The two cartilages come more and more into a horizontal line when viewed laterally. The articular notch on the jaw formerly faced backwards and downwards and now faces upwards; it passes back by the stretching (?) of the jaw until it lies far back under the auditory capsule. At the same time the front end of the jaw is withdrawn more under the nasal capsule.

Before the metamorphosis of the quadrate begins the antorbital process is formed from the anterior edge of the commissura close against the side wall of the skull. Its anterior and upper borders are continuous with the nasal roof and side wall. Its main base of attachment to the root of the commissura lies ventral to nerve V_1. Its anterior end develops a forwardly directed processus maxillaris anterior and its posterior outer corner develops a backwardly, and inwardly, directed processus maxillaris posterior.

Meanwhile the cartilage of the following structures has been softened: the supra-rostral cartilage, the outer ends of the trabecular horns, the posterior part of the quadrate cartilage with the ascending process on its inner, and the tip of the tadpole otic process on its outer, side; and the upper edge of the muscular process. In addition, the subocular shelf is being removed from the trabecular and, with it, the processus pseudo-pterygoideus is being destroyed and the back of the upper part of the commissura softened. These all appear to be simultaneous processes (they may prove to be due to some hormone release).

By some agency unknown the whole quadrate bar is now moved backward, rotating on the commissura, which is therefore itself rotated backward. The quadrate is still attached to the trabecular horn by the ligamentum quadrato-ethmoidale and by the 'trabecular quadrate ligament'. The trabecular horn, and the nasal capsule above it, are therefore rotated downward and backward; the outer half of this horn, and the supra-rostral cartilage in front of it, are entirely destroyed and leave no trace in the adult. The cartilages of the nasal capsule are at this time rapidly developing, fusing with one another and becoming chondrified,

The middle section of the ascending process is entirely

destroyed, leaving temporary stumps attached to the cranial wall medially and to the quadrate laterally; even these are very soon entirely destroyed, so that the ascending process forms no structure in the adult skull.

The backward movement of the softened quadrate bar against the rigid auditory capsule behind, causes the bar to fold on itself in gentle undulations; the outer tip of the tadpole otic process is bent backward, but offers sufficient resistance to prevent the collapse of the quadrate back on to the face of the auditory capsule. Appearances in sections lead one to suppose that the mandibular muscles may play a part in this initial buckling.

Meanwhile the pseudobasal process is condensing as mesenchyme in front of the antero-lateral corner of the auditory capsule. Unlike Gaupp, I can find no indication that it owes its origin in any way to the quadrate; it is rather related to the auditory capsule, especially laterally, where no perichondrium intervenes between the two structures.

These various changes continue rapidly. The upper part of the commissura is destroyed from behind forward and the residue is rotated backward. Its base of attachment to the side wall of the skull and the root of the trabecular horn is usurped by the inward and backward growth of the root of the antorbital process. From the lower outer part of the commissura the pterygoid process of the adult now emerges. The residue of the commissura is eroded from its upper, inner, surface and, below, the angle which it makes with the quadrate bar is opened by further cartilage erosion. This destruction, coupled with a similar destruction laterally, below the muscular process, narrows the transverse width of the quadrate bar in the region above the pars articularis.

In front, the antorbital process has increased in size and has been rotated backward; it also stands out more laterally. Its processus maxillaris posterior has increased in length, and is now fused at its inner end with the tip of the processus quadratoethmoidalis of the pterygoid process. This element of the antorbital process grows greatly in length, becomes straightened, and so forms the anterior half of the adult cartilage upper jaw.

The processus quadrato-ethmoidalis and the pterygoid process behind it show very little growth in length.

The upper edge of the muscular process is turned outward and downward and is finally destroyed. The height of the process is so reduced to a third; in the subsequent buckling the posterior half of the process is much deformed and is practically destroyed. Only the lower anterior part of the tadpole muscular process, therefore, remains to the adult stage: this part becomes the adult otic process of the quadrate.

The backward movement of the quadrate bar causes the undulations of cartilage behind to collapse on one another; they are piled up with the more posterior folds dorsally. All but the perichondrium of these folds is soon destroyed, a statement which applies also to the front part of the tadpole otic process, which is now in contact with the quadrate only through perichondrium. The basal part of the process, however, becomes extended backward to join with a (tadpole) crista parotica which is formed by an expansion of the auditory capsule. The surface of this crista tends to lose its perichondrium and its cells become more or less continuous with a cloud of mesenchyme cells which surround it on all sides and stretch forward laterally to the muscular process in front.

Meanwhile, in front of the notch for the articulation of the ceratohyal, the quadrate bar is turning downward and backward so that it sets more vertically. This is the 'pars metapterygoidea' of Parker and Gaupp and is all of the quadrate of the tadpole which is retained into the adult stage (with the exception of certain quadrate debris to be described). As a consequence of this rotation the notch for the hyoid is beginning to close on itself. Behind the notch the remaining unbuckled part of the quadrate is beginning to fold into a single large U which soon collapses on itself and comes to lie behind, and somewhat above, the pars metapterygoidea. This folded part of the quadrate is then much eroded and its perichondrium falls forward and embraces the inwardly sloping upper edge of the pars metapterygoidea between the muscular process laterally and the pseudobasal process medially. With the collapse of this U the muscular process has passed back, and has been raised upwards

and is now forced backward under the overhanging crista of the auditory capsule, whose anterior outer edge is carried inward in a fold below itself. The notch for the hyoid has now fully collapsed.

Meanwhile the pseudobasal process has become more dense and has been moulded by the pressure of the quadrate upon it. For a time it seems to prevent the total occlusion of the cranio-quadrate passage above it, but later the debris of the quadrate are forced back into the passage against the face of the auditory capsule. When it takes up this most posterior position the curve of the quadrate—where the base of the pterygoid process swings laterally into the pars metapterygoidea—cuts into the soft tissue of the pseudobasal process and begins to fuse with its lateral edge; more medially, the two structures are parted by the inclusion, in the joint, of the pterygoid bone which is developing on the side of the pterygoid process and the inner edge of the quadrate. The pseudobasal process also fuses with the debris of the quadrate bar which lie along the top edge of the pars metapterygoidea; it gives the appearance, therefore, of having grown up laterally to pass into the tissue of the otic process, but this is not the case.

The otic process, so far as the quadrate is concerned, is made out of the undestroyed cartilage of the tadpole muscular process. It is fused to the crista parotica, (a) by the conversion of the q u a d r a t e debris behind it (= lateral folded part of the quadrate bar, posterior part of muscular process, and anterior perichondrium of the tadpole otic process) into cartilage; and (b) by the fusion along its outer upper edge of a rod of cartilage which has formed from the cloud of mesenchyme previously mentioned. This rod is indistinguishably fused with the medial (debris) cartilage, but stands out clearly (in transverse sections) where it lies beside the upper edge of the muscular process more anteriorly. Posteriorly it has fused on all sides with the tadpole crista parotica and now extends this structure outward and downward. All these elements of the otic articulation later become fully fused up.

The remaining quadrate bar continues to rotate backwards, a process which at first alters the inclination of the crista and

in later stages causes an actual bending of the bar itself across the rigid fulcrum of the now fully chondrified pseudobasal process. The back of the pars articularis has meanwhile obtained a large posterior spur which is formed from an independent condensation of cartilage cells which formed behind it and soon fused with it.

During the backward movement of the quadrate bar the anterior face of the pars metapterygoidea has changed its inclination. In early stages it sloped in such a way that it faced forward and i n w a r d towards the side wall of the skull. In later stages it comes to face directly forwards and in the adult it faces outwards and forwards. This movement assisted the root of the pterygoid process to emerge from the quadrate bar and enhances what is, in fact, probably only an appearance of growth in length in this structure. This twisting of the bar greatly alters the appearances seen in transverse sections, on the one hand of recently metamorphosed frogs and on the other of adult animals; it is also responsible for the outward rotation of the tip of the pterygoid process into its adult (lateral) position.

6. Discussion.

An attempt will here be made to analyse the facts of jaw metamorphosis to see what light can be thrown on the peculiarities of structure and attachment of the jaws in the tadpole and the adult frog and to relate these peculiarities to the more normal structures of other groups. It may be mentioned here that there is a great constancy of structure among most of the Anura in the relations of the jaws to the skull, and in allied structures. We are largely indebted for our knowledge of the skull in the group as a whole to Parker's third paper on the Batrachia (1881). But it may well be that more extensive work will show that this constancy is not so great as now appears. Already the work of de Villiers, Wagner, and van Seters has shown that Ascaphus and Liopelma are very far from the common Anuran type in certain points of jaw suspension, while Alytes, Bombinator (Luther, 1924, fig. 20), and Discoglossus (Pusey) also retain certain characters which must be looked upon as primitive from this point of view. Nevertheless, on

our present knowledge, we may assume that the description of jaw metamorphosis here given for Rana temporaria applies, at least in broad outline, to most other Anurans. There is, however, but little comparative evidence of this metamorphosis from other species, and the similarity has rather to be inferred from a few scattered observations, and from the fact that tadpole skulls of essentially similar construction give similar adult skulls. Our evidence on this point comprises the work of Parker on several species, Litzelmann on Bombinator, Kotthaus on Xenopus, and Kruijtzer on Megalophrys and Alytes.

(a) Perhaps the most obvious fact to be noticed in the study of the above reconstructions is that the anterior lower third of the tadpole muscular process becomes the otic process of the adult quadrate. Though both Parker and Gaupp noticed that the adult otic process carried traces of the residue of the muscular process, neither fully realized that these processes are one and the same structure. Litzelmann's figures show the same for Bombinator. In the tadpole skull, then, we may say that the lower front border of the muscular process is the same structure as the adult otic process. When this is established it can then be said that the upper part of the tadpole muscular process is a specialization of the upper part of the adult otic process—a specialization to give increased muscular attachment, and one which is destroyed in the adult. Such a specialization is peculiar only to the Anura. Gaupp considered the whole muscular process to be such a special larval feature, but we may now say that it is only the upper part of the process which is an exaggerated adult otic process. The hinder border of the muscular process in the tadpole slopes downwards and backwards and passes insensibly into the outer edge of the quadrate bar. In the auditory region this outer edge is continued backward to fuse with the auditory capsule as the 'tadpole otic process'. It would seem reasonable then to believe that in the tadpole the anterior edge of the muscular process is the front border of an otic process which, behind, reaches right back along the border of the quadrate bar to its attachment with the auditory capsule

in the tadpole otic process; that is to say, that a shorter otic process in the ancestor has, in phylogeny, been stretched forward to the front of the head, whilst its anterior upper border has been extended upward to give the muscular process.

At metamorphosis the top of the muscular process is destroyed and the outer edge of the quadrate bar becomes buckled and folded on itself and is then impacted between what remains of the muscular process and what remains of the tadpole otic process. The debris so formed—themselves of otic-process origin—are then reconverted into cartilage to assist in the fusion of the two ends of a previous single structure. The lateral band of binding cartilage which is fused on to this joint laterally seems to owe its origin rather to the lateral wall of the auditory capsule, and since it gives rise to the crista parotica of the adult and was already in continuity with the tadpole representative of that structure in its origin, it would seem to be fair to call it the crista parotica, or a specialization of it formed probably to assist in the rapid attachment of the adult otic process. Another view has been put forward by Kruijtzer for an apparently similar structure in Megalophrys and in Alytes; he believes it to be a 'laterohyal' of hyoid origin; this view will be discussed later. Van Kampen has found the same structure in Pipa.

In the view put forward in this paper, then, the whole outer border of the quadrate bar, from the edge of the auditory capsule to the anterior edge of the muscular process, is to be looked upon as a single very much stretched otic process; the tadpole otic process would then be the original connexion of this process with the auditory capsule.[1] At metamorphosis the effect of the stretching during phylogeny is reversed by a process of telescoping in ontogeny. The middle of the process is necessarily weakened and partially destroyed and the debris are rechondrified to join up the anterior and posterior limits into a shorter more typical process again fused to the auditory capsule through what remains of the base of the tadpole otic attachment. The joint is then made stronger by a specialization

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[1] However, Alytes (Kruijtzer) and Discoglossus (Pusey), both primitive frogs, are without 'tadpole otic processes'.

and forward growth and fusion of the crista parotica laterally to the muscular process.

If this interpretation is correct the question does not arise as to which of two otic processes is the homologue of the single otic process of other animals. For the attachment to the skull is always through the base of the tadpole otic process. The fusion of the adult otic process with it is but the completion of the reduction in length of a single process which was very much elongated in the larva. Gaupp was of the opinion that the second fusion of the quadrate with the auditory capsule was the homologue of the otic process of the Urodeles. He considered the tadpole otic process to be a larval specialization confined only to the Anura.

In support of this view of the phylogenetic stretching of a single otic process, the following facts may be brought forward: (1) The rudiment of the annulus tympanicus is found in stage 2 at the base of the muscular process; it passes back with this process during metamorphosis to the auditory region. (2) The anterior (morphologically outer) end of the Eustachian tube reaches to the same point and also moves back at metamorphosis. (3) The same is true of the rudiment of the columella auris. (4) The jaw muscles inserted on the muscular process, especially on its inner surface, pass back to the auditory region without change of attachment. (5) The squamosal rudiment forms on the base of the muscular process in stage 2 and grows upward to about a third of its height; that is to the top of that part which becomes the adult process, and remains so placed into the adult. (6) Finally the forward position of the pars articularis and of the lower jaw, and of the ceratohyal which lies immediately below—morphologically just posterior to the muscular process—all point to the fact that the anterior edge of a single otic process has been carried forward and has taken with it all those structures normally associated with it.

(b) The homology of the ascending process of the tadpole with that of the Urodeles and other forms, has not been in doubt since its true nature was established by Stöhr in 1881. Its relations to the branches of the Vth and VIIth nerves and to neighbouring blood-vessels are correct for such a process.

It is only surprising that a structure so common in skulls of other forms should be entirely absent in that of the a d u l t frog. It may, however, persist in part in the adult skull of A s c a p h u s (see the subsequent discussion, pp. 534 and 535).

(c) The homology of the commissura quadrato-cranialis anterior is by no means so clear. In 1893 Gaupp was led to the view that it represented the connexion found in the salmon between the palatopterygoid bar of the quadrate and the antorbital process of the nasal capsule. In 1906 he was in greater doubt about this structure and stated that it had no 'analogue' in the U r o d e l a. In 1925 Edgeworth made a survey of the occurrence of an attachment of the tip of the pterygoid process of U r o d e l e s to the trabecular wall of the cranium below the later-developed antorbital process. As a result of his researches he said:

'These phenomena indicate an ancestral Urodelan condition in which the pterygoid process was continuous anteriorly with the trabecula, i.e. one in which there was a pterygoquadrate. Gaupp held that the commissura quadrato-cranialis anterior of the larval stage of R a n a has no 'analogue' in the U r o d e l a, but the new information above mentioned shows that it is homologous with the pterygoid process of U r o d e l a and should be similarly named. The later developed pterygoid process of Gaupp might have a new name.'

A number of criticisms may be raised against this view. Firstly, Edgeworth is forced by it to believe that the frog's pterygoid process, which itself is formed from the distal part of the commissura, is not homologous with that of U r o d e l e s. Apart from his hypothesis there seems to be no other obstacle in the way of this latter homology; he therefore solves one difficulty only to supply a new one. His view is that the tip of the pterygoid process in both young U r o d e l e s and in the tadpole is fused to the trabecula below the antorbital process; in the U r o d e l e s by tissue which is subsequently destroyed, and in the tadpole by the commissura which is also destroyed. In some adult U r o d e l e s there is a cartilaginous connexion of the tip of the pterygoid process with the antorbital process as in the adult frog. From his figures and descriptions I am left

in doubt as to how far these two connexions in the Urodeles are to be considered as separate.

But his view seems to be untenable since the processus quadrato-ethmoidalis—which on non-Edgeworth homologies becomes the tip of the pterygoid process—projects forward from the front face of the commissura. So that here we have part of the pterygoid process beyond the fusion of the commissura with the skull. However, Edgeworth disposes of this difficulty by disowning the subsequently developed pterygoid process of the frog and saying that a new name should be found for it; he offers no homology for it.

(d) If there are two possible connexions of the pterygoid process to the skull in Urodeles as Edgeworth suggests, (1) to the root of the antorbital process, and (2) to its processus maxillaris posterior, only the latter seems to be represented, in the frog, in the adult. The processus quadrato-ethmoidalis is admittedly continued forward by a ligament—ligamentum quadrato-ethmoidale—to the side of the trabecula, but this cannot represent connexion 1 above, of the Urodele, as both the process and the ligament lie laterally to the inner nostril and the ligament joins the trabecula in front of it. In the Urodeles this connexion must lie behind and therefore medially to the nostril. So that even if we do not accept Edgeworth's homology, if we accept his facts, we are still left with a connexion in the Urodeles unparalleled in the frogs. And the ligament of the frogs is not found in the Urodeles. This ligament may probably be explained as a specialization of the rudiment of the pterygoid bone. On becoming detached from the trabecula, its hinder part ossifies as that part of the pterygoid which lies medially to, and overlaps, the maxilla. However, it seems to me that this difficulty is not greater than that left unfaced by Edgeworth, namely of finding a homologue in the Urodele (or in any other animal) for the cartilages which give the apparent pterygoid process of the frog.

Gaupp's view also raises difficulties. He believed that the commissura of the frog was the whole palatopterygoid bar shortened up and joined to the cranial wall by the fusion of the ethmopalatine-articulation seen in the salmon (de Beer, 1927).

The processus quadrato-ethmoidalis then represented that part of the palatine bar in front of this hinder articulation. The great growth of the processus maxillaris posterior of the antorbital process was then to be considered as a special anuran modification. However, the whole pterygoid bar up to the antorbital process seems to belong to the quadrate in those Urodeles which retain it (i.e. in Ranodon and, perhaps, Hynobius and Menopoma; Edgeworth, 1925). In the salmon we find the anterior end of the palato-pterygoid bar chondrifying separately from the part behind (de Beer, 1927). The facts then from the Urodeles and the salmon suggest that the processus maxillaris posterior (which, in the frog, belongs to the antorbital process) is really a detached front end of the palato-pterygoid bar secondarily attached to and developing from the antorbital process. Such a view cannot be made to agree with that of Gaupp.

(e) An alternative interpretation will now be put forward, which is not concerned with these difficulties.

The descriptions given of these parts in the tadpole show that the adult frog pterygoid process emerges from the distal part of the commissura where the latter passes into the quadrate bar. To this pterygoid process is added the small forwardly projecting processus quadrato-ethmoidalis which projects forward from the middle of the front face of the commissura; together these two cartilages make up the adult pterygoid bar. On the medial and upper side of this bar, then, there lies the remainder of the commissura—that part which will subsequently be detached from the skull wall and destroyed. When so detached—had it not been so fully eroded and bent so that it points forward—it would lie as an inwardly directed process of the quadrate on the inner side of the base of the pterygoid bar close to the point where this swings laterally into the body of the adult quadrate bar. It would, in fact, have all the relations to the quadrate and the pterygoid process of a basal process. The view here put forward then is that the commissura is the basal process of the quadrate which has passed exceptionally far forward to fuse with the trabecula just behind the nasal

sac. Compare the conditions described in Scyllium (de Beer, 1931). It may be assumed that in phylogeny it ceased to abut against a basitrabecular process from the cranial floor and passed progressively forward, probably in subsequent ontogenies, fusing with the edge of the trabecula. In the tadpole it has taken up the most anterior position possible—as here it is stopped by the nasal sac and its inner nostril to which the commissura makes a posterior border.

The relations of this attachment to surrounding structures must now be examined. It should lie dorsally to nerve VII palatine and ventrally to nerve V_1; it does so. Other structures such as the lateral head vein and the carotid artery do not reach far enough forward to assist us. But it may here be noted that there seem to be no obstacles in the way of such a forward movement. In addition, as shown above, the anterior border of the otic process (tadpole muscular process) lies opposite it on the outer side of the quadrate bar. It also bears the correct relations to the pars articularis of the quadrate and the rudiment of the pterygoid bone. Much of this evidence is of a negative nature, but later it will be shown that the conditions found in Ascaphus and Liopelma probably may support this view.

(f) It will be assumed then that the commissura is the basal process of the quadrate. In that case the 'pseudobasal process' cannot also be the basal process. Gaupp believed that it was, and his view has been accepted by most other workers up to the present. What then is the pseudo-basal process? From the study of its origin Gaupp found it hard to believe that it belonged to the quadrate; however, in a 37 mm. embryo he said that he found a connexion. I cannot confirm this point. Rather, as Gaupp had to admit, it seems to belong to the lateral anterior face of the auditory capsule in front of the foramen ovale. Kruijtzer has described the independent origin of this structure in Megalophrys and Alytes, and its secondary fusion to the quadrate. In this region its cells are continuous with those of the capsule, from which, laterally, it is not separated by any perichondrium during development. Later, however, it becomes separated from the capsule by a fibrous

joint and it becomes fused to the quadrate—though at a point far from the region of the quadrate from which Gaupp suggested that it might be developed. Gaupp was impressed by the similarity of the adult process to the basal process of the Urodele quadrate, and almost against his own evidence he was forced to advocate this homology. Since then, however, the nature of the articulation of this process with the capsule has been called in question by de Beer (1926).

In this paper it is assumed that this 'pseudobasal process' of the adult frog is the outer end of the basitrabecular process from the basal plate which has been separated off from the cranial floor by the destruction of its root. It is suggested that it has become fused secondarily to the quadrate following the complete destruction of the true basal process (commissura) and also secondarily has obtained a jointed articulation with the auditory capsule, thus restoring a more Urodele-like arrangement in this region. The reasons for this belief will be discussed when the skulls of Ascaphus, Liopelma, and Alytes have been described (see p. 539). It should here be noted that had the commissura not been distorted and destroyed during metamorphosis in Rana, but had merely been detached at its upper end from the skull, it would have abutted against the pseudobasal process, making thus a joint between a true quadrate basal process and the end of a cranial basitrabecular process (albeit detached).[1]

Little mention need be made of the other processes of the jaw apparatus. The antorbital process is undoubtedly the homologue of the structure found in other animals. Only its processus maxillaris posterior seems to be unusually well developed as a unique elongated rod making up about half the cartilage upper jaw in the adult. The pterygoid process has been already dealt with.

(g) A summary may here be given of the homologies put forward in this paper:

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[1] Since writing this account I have found a small basal process in tadpoles of Discoglossus before metamorphosis; it lies below the ascending process in the typical vertebrate position; its presence probably does not invalidate the present argument (Pusey).

1. The commissura quadrato-cranialis anterior is the basal process of the quadrate.

2. The whole outer border of the quadrate bar from the anterior edge of the muscular process to the tadpole otic process is the single much elongated otic process of other forms. The middle of its length only is destroyed and so it collapses on itself to give a shortened adult otic process.

3. The lower anterior part of the muscular process gives the cartilage which forms the adult otic process. The remainder is a unique specialization found only in the Anura.

4. The 'pseudobasal process' (= basal process of Gaupp) is the isolated outer end of the basitrabecular process of the skull plus post-palatine commissure behind (see pp. 529 and 539).

5. The processus quadrato-ethmoidalis and the pterygoid process (from the commissura) are together equal to the Urodele pterygoid process.

6. The ascending process and the antorbital process are homologous to the similar structures in other forms.

7. The excessive length of the processus maxillaris posterior is either a unique specialization of the Anura, or the detached front end of the palatopterygoid bar.

8. The ligamentum quadrato-ethmoidale is an early specialization of the rudiment of the pterygoid bone.

9. The 'trabecular-quadrate ligament' is a special Anuran feature; its hinder part ossifies as the quadrato jugal.

Apart, then, from a possible additional attachment in the Urodeles between the tip of the pterygoid process and the trabecula, below the antorbital process, we find a close agreement between the parts found in the Urodeles and the Anura. The differences in the relations of the Vth and VIIth nerves in the two groups will be discussed later in this paper.

7. A Redescription of the Temporal Regions of the Skulls of Ascaphus, Liopelma, and Alytes.

By far the most important work on the Anura in respect of the jaw anatomy and the temporal region has been the descriptions recently given of the adult structure of single specimens of Ascaphus and Liopelma. These two species

are placed in a separate family—the Liopelmidae—by Noble, who appears to consider them to be the most primitive living Anurans; other workers have placed them with the Discoglossidae. So far as the temporal region of their skulls is concerned, it seems very reasonable to agree with Noble's views.

(a) Ascaphus truei.

The account here given of the temporal anatomy of Ascaphus is taken from the work of de Villiers (1934). His published pictures (fig. 7 A, B, C, and D), together with two drawings taken more anteriorly and most kindly supplied to me by Miss de Vos of Stellenbosch, have been reconstructed by the method previously described. These reconstructions (figs. 26 and 27, Pl. 45) are seen from in front. In fig. 27, Pl. 45, much of the quadrate has been removed and in both figures the part above the pars articularis had been drawn in freehand (hence in dotted lines) by analogy with Rana and Liopelma; however, the position of the lower jaw is known to be correct from fig. 7 D. In fig. 27, Pl. 45, the nerves are shown. The present author is solely responsible for any differences of description or nomenclature here introduced and for any errors brought in the reconstructions.

De Villiers draws attention to the Urodele-like character of the nerve foramina and arrangements generally of the temporal region. The facial nerve is separated from the trigeminal nerve; some part of the separating cartilage must represent a prefacial commissure—a structure entirely absent in Rana and the bulk of frogs. In addition, the foramen for the trigeminal nerve is divided into an upper and a lower division by a bony bridge (tb.) in front. From the upper opening nerves V_2 and V_3 pass out, and from the lower opening V_1, a nerve connecting V to VII palatine, and the lateral head vein; these latter two structures are not shown in fig. 27, Pl. 45, but the main branches of nerve V and the divided trigeminal foramen are clearly shown.

The VIIth nerve ganglion (fig. 27, Pl. 45, VII g) lies in a foramen ventral and lateral to the trigeminal foramina; from it the palatine branch of VII passes forwards (VII p.). VII

hyomandibular is separated from VII palatine by a skeletal bridge behind which it runs out laterally through its own foramen, which is situated between the auditory capsule above and 'a ventral cartilage ledge of the auditory capsule' below. This ledge is carried out laterally to the auditory capsule and, behind, is developed into a hook which articulates with the hyoid cornu; VII hyomandibular runs out over this ledge, gives off two branches to the IXth nerve, and then runs down behind and alongside the hyoid cornu.

The root of the Vth nerve runs in the cranial cavity medially to the functional inner wall of the auditory capsule; but the root of the VIIth nerve runs at first with the VIIIth and then passes through the foramen acusticum anterius and forward in the cavity of auditory capsule to its ganglion in the palatine foramen.

The pterygo-quadrate complex is peculiar in that it carries a median process (fig. 26, Pl. 45, *bpr.*?) on the inner side of the root of the pterygoid bar (*ptc.*) where the latter passes into the body of the quadrate. This process is a thin plate which abuts against the antero-lateral floor of the auditory capsule and underlies the front part of the 'ventral cartilage ledge' mentioned above. It lies in front of the palatine branch of the VII. The outer upper part of the quadrate carries an upstanding process (fig. 26, Pl. 45, *pmq.*?).

In the absence of any embryological evidence we can only interpret these structures by an analogy (if not a homology) with the Urodele condition (see Goodrich, 1930, pp. 259 and 278). Firstly, since the root of the VIIth nerve runs in the cavity of the auditory capsule, we may assume that the true floor of the capsule is missing.[1] That being so, the acting floor to the capsule must be a lateral outgrowth from the basal plate of the skull floor, with which the capsule has become secondarily fused. Clearly part of this lateral outgrowth lies in front of the palatine branch of VII (de Villiers, 1934; fig. 7 c and D); it is therefore to be considered as a true

[1] This is supported by my own unpublished observations on Discoglossus; here the ventral capsular floor above the VIIth nerve develops, but only very late.

basitrabecular process (*bt.*?); part lies behind this nerve and is a lateral commissure. The capsule has presumably grown down laterally as well as medially on to this plate as in Urodeles, thus separating nerve VII palatine from VII hyomandibular. The acting median wall of the capsule—since it separates the root of the Vth nerve from the root of the VIIth nerve—is a pre-facial commissure; no such structure has previously been so named in the Anura, though van Seters has described a similar state of affairs in Alytes (van Seters, 1922). A prefacial commissure also occurs in Bombinator (Luther, 1924, fig. 20) and in Discoglossus (Pusey); de Beer (1926) remarks that it is absent in the Anura. The conditions in this region, then, are almost identical with those of Urodeles.

In discussing the trigeminal bridge (*tb.*), and the separation of the trigeminal and facial foramina, de Villiers says: 'One is inclined to consider this condition primitive, but it is equally possible that it may be a case of partial neoteny, as a separation of the facial from the trigeminal foramen is recapitulated in Anuran ontogeny.' As it stands, this statement appears to me to be untrue; though he does not say so, he is presumably referring to the separation of nerves V_2 and V_3 above, from nerves V_1 and VII palatine and hyomandibular below, by the ascending process in the tadpole. This separation is not complete, in that the outer part of the ascending process is not fused to the front face of the auditory capsule. Were this the case, however, and should this bridge remain intact into the adult, we should have a condition as shown by the trigeminal bridge in Ascaphus. Only embryological evidence can show whether this is the true nature of this bridge, as may well be the case. [From the reconstructions it may be seen that the attachment of the ascending process would then have been made to the pila pro-otica well above the level of the trabecula itself; such a high-level attachment occurs in Alytes (van Seters)[1] and in Urodeles.] But the ascending process can never separate off all the branches of the Vth nerve from the branches of VIIth as de Villiers seems to suggest. Only a prefacial commissure is able to separate the root of V from the root of

[1] Also in Discoglossus (Pusey).

VII. We have in Ascaphus not one bridge but two in this region—the upper, possibly the inner end of the ascending process secondarily fused laterally to the front face of the auditory capsule; the lower, a prefacial commissure. It seems probable that this arrangement is primitive; but de Villiers's apparent contention that the retention of an ascending process into the adult is neotenic might be correct did it occur in Rana and the modern type Anuran, but this retention must have been primitive in the early Anuran ancestor (and possibly in Ascaphus too) where the presence of the ascending process in the adult would have been the normal state of affairs—as in the Urodeles to-day.

In the absence of embryological evidence we may assume that Ascaphus has a basitrabecular process. Against it abuts a median process from the quadrate—giving therefore an articulation typical of Urodeles and other forms. This quadrate process can only be a true 'basal process' therefore; de Villiers has marked it so in his figures; but in his other works he also marks the pseudobasal process as a basal process. The discussion of this point will be deferred. It is to be noticed too that the quadrate carries an upstanding process; this may well be the muscular process of the larva and has been marked *pmq.*? in fig. 26, Pl. 45. The actual otic attachment is then made out of a more median part of the quadrate bar than is the case in Rana.

(*b*) Liopelma hochstetteri.

This description is taken from the two papers of Wagner, 1934, and from a study of the reconstructions here made from her published pictures. (These pictures were found to contain certain errors. In her second paper the order of the figured sections should be A, C, B, and not A, B, C, in fig. 8; and the structure marked *aso.* should have been marked *prbs.* in fig. 8A; these corrections were very kindly confirmed for me on the original sections by Miss de Vos of Stellenbosch.)

The root of the Vth nerve is parted from that of the VIIth by a cartilage bridge (figs. 28 and 29, Pl. 45, *pfc.*). (In her figures Wagner does not show nerve V_1 as her sections do not

go far enough forward; in fig. 29, Pl. 45, it has been put in by analogy with other frogs; it is marked V_1?). The trigeminal foramen is undivided. The root of nerve VII does not run in the cavity of the auditory capsule in passing to its geniculate ganglion. This ganglion lies in a groove in the front face of the auditory capsule (fig. 28, Pl. 45, *ff*.). The groove is roofed by the overhanging auditory capsule (*ac.*) laterally, and by what must be a prefacial commissure medially (fig. 28, Pl. 45, *pfc.*). Its floor is made up of a ledge which runs out from the floor of the skull and passes laterally to project beyond the auditory capsule at the side (*lac.*); the capsule is fused downward on to it laterally to the facial ganglion. Nerve VII hyomandibular runs backward and laterally from the ganglion, behind part of this fused bridge, as in Ascaphus; it passes out of its own foramen between the ledge below and the auditory capsule above, and runs back down the hind face of the hyoid cornu, after giving off a branch to nerve IX (see fig. 28, Pl. 45, VII *hm.*, IX *rc.*). The hyoid cornu is fused with the hinder lateral margin of the ledge (fig. 29, Pl. 45, *ch.*).

The palatal branch of nerve VII passes forward from the ganglion. It is only separated from the branches of nerve V by the prefacial commissure (*pfc.*); consequently there is no basitrabecular process in front of it. In addition, the facial ganglion lies more medially than in Ascaphus. Wagner shows a considerable notch behind VII palatine in her fig. 8 B—a notch which appears to be bridged across by dense mesenchyme.

If we use the method of interpretation by analogy as for Ascaphus, we note the presence of a prefacial commissure, the absence of a basitrabecular process, but the presence of a large post-palatine commissure (the ledge, *lac.*, behind and below the facial ganglion) which is continued far forward, laterally to the ganglion and the palatine nerve. The auditory capsule has its own floor above the commissure.

The pterygo-quadrate is just as in Ascaphus. It has a large flat basal process (which here rests on the lateral ledge, *lac.*, and not under it as in Ascaphus) which arises from the inner side of the base of the pterygoid process. The ? muscular

process (*pmq.*?) is not carried up into such a high ridge as in Ascaphus.

(*c*) Alytes obstetricans.

This description is taken from van Seters's account of the larval skull and from the reconstruction here made from his figures (*a–h* of his fig. 3). This reconstruction (fig. 30, Pl. 45) has been reversed for comparison with Ascaphus and Liopelma.

The roots of the Vth and VIIth nerves are separated by a plate (fig. 30, Pl. 45, *pfc.*) which van Seters calls the 'cloison médiale de la capsule auditive'; this can only be a prefacial commissure and therefore part of the side wall of the skull and not part of the auditory capsule. The root of nerve VII passes through the auditory capsule for a very short distance before passing under the true ventral floor of the capsule (= 'une petite cloison oblique', van Seters) into a tunnel whose floor must represent a post-palatine commissure (= 'une partie horizontale, provenant de la paroi ventrale de la capsule auditive et du plan basal'). It then passes out of the mouth of this tunnel below the trigeminal foramen which lies above it. Apparently the trigeminal and facial ganglia are partly fused into a ganglion pro-oticum in front of these foramina. The VIIth hyomandibular nerve passes to the side after the root of VII has left its tunnel; though it is not shown in the figures, VII palatine must also pass ventrally after VII has left its tunnel. There can therefore be no basitrabecular process in front of the palatine. How far laterally the post-palatine commissure may stretch is not described.[1]

In the adult Alytes, Kruijtzer finds the pseudobasal process (his pharyngohyal) as an independent knob of cartilage just in front of the foramen ovale, as in Rana. The VIIth nerve takes the typical anuran course in relation to it.

Fig. 31, Pl. 45, shows an anterior view of the temporal region of a young adult Rana with the main branches of nerves V, VII (and some of IX) put in, for comparison with the frogs

[1] The nature of these structures is fully confirmed by my work on Discoglossus (Pusey).

described above. In Rana, of course, there is no prefacial commissure, so that the branches of V and VII are not parted, and all emerge from the single ganglion pro-oticum (*gp.*) in the foramen pro-oticum (*fp.*).

8. DISCUSSION.

It seems impossible to doubt that the temporal arrangement of the skull of Ascaphus is primitive and shows the original morphology of this part of the skull in the ancestral frogs. It is a striking fact that not only does this frog contain a full complement of those structures usually found in this region in other groups—i.e. basitrabecular process, post-palatine commissure, prefacial commissure, and possibly part of the ascending process—but has them specialized in just the peculiar manner found in the Urodele skull, i.e. with the auditory capsule carried far forward over the VIIth nerve and fused with the prefacial commissure and basitrabecular process below it, so that VII palatine is parted from VII hyomandibular. With a knowledge, therefore, of the anatomy of Ascaphus it becomes far more easy to trace the morphology, on the one hand of the Urodeles, and on the other of the more specialized frogs, back to a common ancestral type. This is of importance in that it must necessarily reduce one of the arguments of those who would derive the frogs and the Urodeles independently from the fish (Holmgren, 1934, and Säve-Södebergh, 1934 and 1935). The evidence seems rather to strengthen what may be called the classical view of the common origin of these two groups within the Amphibia.

(*a*) In the absence of adequate palaeontological evidence on the problem of the origin of the Anura it will be interesting from a study of comparative anatomy to see how the Ranid type of temporal region must have been derived from such a condition as that shown in Ascaphus. A comparison of figs. 27 and 31, Pl. 45, will show what changes are necessary. The changes consist in the collecting together of all the branches both of nerves V and VII into a single pro-otic foramen. This can only be done, in effect, first by pulling VII palatine through the basitrabecular process in front of it. It is to be noticed

that this is the stage of development shown by Liopelma [if, in addition, the trigeminal bridge of Ascaphus (ascending process?) is also destroyed].

This change can be made in two ways: (1) by destroying all of the basitrabecular portion of the 'lateral cartilage ledge' from the basal plate and leaving only the post-palatine portion behind it; or (2) by destroying only the root of the basitrabecular process and leaving its lateral part fused to the post-palatine commissure behind, and its front part projecting forward alongside, and laterally to, the facial ganglion. From a mere anatomical study it is suggested here that Liopelma shows condition number 2. However, whatever its genetic origin, such a block of cartilage lying behind the VIIth palatine nerve becomes, by definition, post-palatine commissure.

(b) The next step towards the Ranid condition will be achieved if nerve VII hyomandibular is pulled through the lateral junction of the auditory capsule with the underlying 'lateral cartilage ledge' in a Liopelmid stage (see fig. 29, Pl. 45). This will bring both VII palatine and hyomandibular into a common facial foramen separated from the trigeminal foramen only by the prefacial commissure. It will also leave the outer end of the 'lateral cartilage ledge' projecting forward freely from the antero-lateral face of the auditory capsule just in front of the foramen ovale; behind, the process will still be fused to the auditory capsule by the post-palatine commissure and its outer posterior corner will still give an attachment to the hyoid cornu. This is the condition found in Alytes and we may now suggest that the forwardly projecting process of the lateral cartilage ledge is represented in Alytes and all other Frogs by what has, in this paper, been called the pseudobasal process. (Gaupp's basal process.) The pseudobasal process would thus be the isolated outer end of the basitrabecular process plus the post-palatine commissure behind it. This suggestion is in agreement with its development in Rana, where the hinder part develops in continuity with the auditory capsule (= post-palatine commissure, therefore) and the front part is a condensation in situ of mesenchyme (= outer end of

the basitrabecular process). The attachment of the hyoid cornu is also identical and so are the relations of the branches of nerve VII.

Finally, to bring the Alytes condition to that of Rana, it is necessary to destroy the prefacial commissure entirely, and so allow the facial and the trigeminal foramina to run into one. [A prefacial commissure occurs also in Bombinator (Van Seters, 1922; Stadmüller, 1931; and Luther, 1924, fig. 20).[1] Parker's figures (1881) seem to show a divided pro-otic foramen in his Hyla and Hylaplesia.]

It should be noted that van Seters sums up the evidence relating to the segmentation of the otic region of the skulls of Anura and Urodeles. He comes to the conclusion that the Anura have two metotic somites while the Urodeles have three. It will be interesting to await investigations on this point in Ascaphus and Liopelma; it may well prove that these two frogs add to their other Urodele-like characters the possession of three metotic segments in the skull; for, like the Urodeles, their auditory capsules undoubtedly reach morphologically farther forward over the facial nerve and in addition they possess three foramina for the entrance of the VIIIth nerve into the capsule—another Urodele feature—as against two in most other frogs. Ascaphus and Liopelma contain three auditory foramina (de Villiers and Wagner); Kruijtzer has found three in Megalophrys, and Miyiwiki (1927) has described the same for a number of oriental frogs. Other known Anurans contain only two. It may well be that the loss of part of the basitrabecular process, &c., may be related to the suppression of the one out of three metotic segments.

So important are Ascaphus and Liopelma for the interpretation of the frog's skull that it is very necessary that their development should be worked out in detail. Detailed work should also be undertaken in the temporal anatomy both of other primitive frogs and also of Urodeles. It may well prove that the presence or absence of such structures as the basitrabecular process, the bridge between it and the auditory capsule above, the prefacial commissure, and the foramen

[1] And in Discoglossus (Pusey).

acusticum medium may be of use in supplying independent evidence of the evolution of the families of frogs within the class Anura.

It is suggested here then that the pseudobasal process of more typical Anurans is of basitrabecular and post-palatine origin and belongs to the skull and not to the quadrate. Following the steps of Ranid development it is suggested that while it develops from the base of the auditory capsule it is only secondarily fused on to the quadrate and only secondarily jointed on to the capsule. Since, then, the pseudobasal process of typical frogs is probably represented by the basitrabecular process of Ascaphus (i.e. its lateral cartilage ledge of the auditory capsule), the process in Ascaphus and Liopelma from the root of the pterygoid process of the quadrate cannot be the pseudobasal process of such frogs. It must then be some other structure altogether. In the absence of any embryological evidence the suggestion is here made that this quadrate process is the commissura of the tadpole which has been detached from the skull without destruction in these Liopelmid frogs, and has been carried back to make a typical basal-basitrabecular articulation behind. It is, in fact, at successive stages of development first the commissura and secondly the basal process.[1] Such a backward movement of a pre-existing larval structure would account for the fact that in Ascaphus it rests below the 'lateral cartilage ledge' and in Liopelma above it, the position it takes up being rather a matter of chance from the morphological standpoint.

This discussion has contained very much unsupported theory, but it is clear that a study of the development of Ascaphus, which has a free-swimming larva, can settle certain points one way or the other. [Liopelma hatches from the egg as a jumping frog (Archey, 1922) and therefore its development may be expected to be less enlightening]. It can settle whether or no the commissura quadrato-cranialis anterior becomes the basal process of Ascaphus and Liopelma. If this is

[1] Discoglossus gives no support to this view. Here the commissura is totally destroyed at metamorphosis, and a pseudobasal process is formed in the usual way (Pusey).

proved to be the case it then proves that the pseudobasal process of other frogs c a n n o t be the basal process of the quadrate as most workers have believed up till now. So, for this reason also, it is imperative that the development and metamorphosis of A s c a p h u s should be worked out. The confirmation of the whole argument here set out awaits this evidence.

(c) There remains for discussion another theory of the nature of the pseudobasal process, a theory which is at variance both with the older view of Gaupp and with that put forward here; it is rather a return to Parker's and Huxley's idea of the hyoid suspension of the jaws.

Kruijtzer, 1931, examined the development of the chondrocrania of Megalophrys montana, and of Alytes. He observed in Megalophrys an independent cartilage nodule between the crista parotica and the (adult) otic process of the palatoquadrate; he called it the 'laterohyal'. In addition, he noted the pseudobasal process as a chondrification independent of the quadrate and connected to the lower edge of the fenestra ovalis; he called this process the 'pharyngohyal'. In Alytes he also found the pseudobasal process as an independent cartilage connected by a ligament to the plectrum of the middle ear apparatus. He also noted a 'laterohyal' as in Megalophrys and found it to be continuous with the pharyngohyal. His idea is that these joined processes are the ventral and dorsal attachments of the hyoid arch to the skull and that the quadrate bar is fused to them in front. Van Kampen in 1926 found an independent 'laterohyal' in Pipa.

We may take it, from their figures and descriptions, that the 'laterohyal' seen by these workers is the same structure which I have described in R a n a—namely, the 'cloud of mesenchyme' from the tadpole crista parotica which chondrifies to extend this shelf laterally and which is fused farther forward along the upper outer edge of the muscular process (adult otic process of the quadrate). The pharyngohyal is equally certainly the pseudobasal process of R a n a. Is the connexion which Kruijtzer has seen between them the band of quadrate cartilage formed from the debris of the buckled quadrate bar behind? for, as I have been at pains to explain, this is of q u a d r a t e

origin in Rana and makes a continuous bridge from the latero-dorsal side of the pseudobasal process along the top of the quadrate bar to the mass of otic cartilage laterally—a mass made up in part of reconstituted quadrate debris and in part of the 'mesenchyme cloud', when this has chondrified. If this is so, as seems probable, then the theory is already weakened in that the middle of the bridge, at least, may be of quadrate origin in these frogs. It may also be pointed out that in Alytes the tadpole otic process is absent (van Seters) and only one case of its occurrence was noted by Kruijtzer in Megalophrys.[1] If then the 'laterohyal' described in these forms is not the equivalent of the mesenchyme cloud in Rana, there remains the possibility that it is the otic process of the tadpole delayed in appearance; in such a case it would be of quadrate or at least of cranial origin. (It may be noted here that Litzelmann's figures of Bombinator show a continuous cartilage bridge from the pseudobasal process to the crista parotica.)

Kruijtzer's theory of the jaw attachment has been built up by the application of the views of Schmahlhausen (1923), van Kampen (1926), and Stadmüller (1931) on the hyoid arch, to the particular problems of the Anura. The chief criticism which can now be brought against it is that it takes no account of the structures found in Ascaphus and Liopelma (the anatomy of these frogs had not been described in 1931 when Kruijtzer formulated his scheme). It appears to break down when applied to them, if the present interpretation of their structure proves to be correct. In addition, the theory seems to introduce additional difficulties into the already difficult problem of finding homologies for the parts of the Anuran auditory cartilages. Kruijtzer believes that the columella of the frog (i.e. the plectrum) is also part of the hyoid arch in addition to his pharygo- and latero-hyals. He is faced, then, with the problem of two dorsal attachments of the hyoid arch to the crista parotica. If he assumes the insertion of the base of the plectrum into the front of the fenestra ovalis to be the ventral attachment of the hyoid arch, he is also faced with a further duplication, in that his pharyngohyal already fulfils this function. The

[1] Discoglossus has no tadpole otic process (Pusey).

settlement of the claims of his view and of the view put forward
in this paper must await further work, particularly on the
development of A s c a p h u s . Both cannot be correct. Violette
(1930), in a short note, has supported the view that the Anuran
plectrum belongs, not to the hyoid arch, but to the first branchial
arch behind it. He has worked on R a n a   p a l u s t r i s and is led
to this theory by a consideration of the relations of the plectrum
and columella to the IXth nerve and its ganglion. On the other
hand, Salvadori (1928) has found a connexion both in B u f o
and in R a n a between the outer end of the columella and the
ceratohyal. Both these workers cannot be correct, though,
should Violette prove his point, the application of his findings
to Kruijtzer's theory would ease the difficulty of finding
homologies. However, his theory seems to be most improbable.
It cuts across all previous work and his own published evidence
is negligible.

My own investigations on the development of columella in
R a n a lead me to the conclusion that it is undoubtedly a hyoid
structure. In such a stage as 3 of my series I find the rudiment
of the columella stretching from the fenestra ovalis almost to
the front edge of the muscular process. It consists of the very
tenuous but quite distinct mesenchyme band—the 'suspensorio
columellar ligament' of other workers—with two condensations
on it, the extra stapedial in front and the columella behind.
Running parallel with it, and close on its inner, morphologically
anterior side lies the Eustachian tube which also stretches
forward toward the muscular process. Somewhat below it and
lateral to it—on its morphologically posterior side—runs the
main branch of nerve VII. It is hard to believe that these
structures do not represent a spiracular pouch with the hyoid
skeleton and nerve behind it.

On the whole, then, it seems that neither Kruijtzer's nor
Violette's theory is tenable. However, more work must be done
on the temporal region of the skulls of both primitive frogs and
U r o d e l e s before the very old and long-disputed question of
the nature of the Anuran pseudobasal process can finally be
settled.

9. The Probable Evolution of the Tadpole Jaw Apparatus.

When once a scheme of homologies has been built up so that all tadpole and frog structures can be homologized with structures in the Urodeles, it is possible to visualize the processes which must have gone to the establishment of the extraordinarily specialized larval arrangement of the jaws in the tadpole.

Clearly the otic process has been very greatly elongated to give the whole lateral border of the quadrate bar from the auditory capsule to the front border of the muscular process; this latter process has, then, been developed as an exaggeration of the otic process to give increased muscular attachment in connexion with a specialized jaw and branchial apparatus. Medially, the basal process has slipped forward and passed along the trabecula to its very advanced position as the tadpole commissura; the movement of these two processes forward has stretched the body of the quadrate into a long flat strap—the quadrate bar of the tadpole; the whole of this must represent the small thick rod found far back in Urodeles. The subocular space between the skull and the quadrate may well have been much smaller in ancestral larval frogs; all that is absolutely necessary is a small foramen for V_1 behind; in Dactylethra (= Xenopus) and in Pipa (Parker, 'Batrachia II') the space is very small. It may be that the subocular shelf on the trabecula and the processus pseudopterygoideus are relics of the cartilage which may have filled this space; apart from this suggestion no other homology can be found for this last peculiar process which is of short duration in the larval life and seems to have no obvious function; in many other frogs it is a much larger structure than in Rana. Gaupp could find no homologue for it among the structures of other groups. The basal process would seem to have increased its base of attachment to the pterygoid process so that this latter becomes masked in the outer part of the commissura above and below by the extension of the pars articularis of the quadrate. As a result, the pterygoid bar must have become useless as an upper jaw cartilage. The trabecular cornu and its probable derivative, the supra-rostral

cartilage, then became the skeleton of the tadpole upper jaw.
With the forward movement of the quadrate the lower jaw
became progressively shortened and therefore would have
become transverse; it became divided into two segments.
Probably the ligaments from the quadrate to the trabecular
horn were evolved in response to the mechanical strains set
up by the very vigorous rasping method of feeding; the liga-
mentum quadrato-ethmoidale would seem to be a specialization
of the pterygoid bone rudiment, while the 'trabecular-quadrate
ligament' seems to be associated with the quadrato-jugal.

Meanwhile the changes in the temporal region would have
gone on independently of this stretching and, as has been
suggested, they may have been due to the suppression of a
metotic segment from the skull.

10. An Interpretation of the Meaning of the
Jaw Metamorphosis.

The metamorphic changes in the jaws of the tadpole seem
to be a reversal of those phylogenetic changes which had brought
about the specialized larval organization. That is to say, those
structures which were stretched during phylogeny are now
hastily reduced again to more typical adult proportions (e.g.
the otic process, the whole quadrate bar, and the muscular
process). Those which had been shortened or obscured are
lengthened or carved out again (the lower jaw and the pterygoid
bar). Those structures which were carried into new positions
return again (commissura?; annulus tympanicus, columella
apparatus, Eustachian tube, and ceratohyal; jaw muscles—
both origins and insertions; nasal cartilages, pterygoid, and
squamosal), whilst those whose development had been sup-
pressed in the tadpole organization are now rapidly reformed
(nasal cartilages, pseudobasal process, crista parotica, and
several bones). Finally, those structures which were developed
purely as larval specializations are destroyed without leaving
a trace (outer ends of the trabecular cornua, the supra-rostral
cartilage, many of the contours of the lower jaw, and the
muscular process, in part). It is not easy to explain the total
destruction of the ascending process. Also the changes in the

temporal regions seem to have gone on independently of the stretching of the quadrate; as suggested, they may be due to a loss of a metotic segment from the skull and the consequent shortening of the auditory capsule. The late development of the basitrabecular process (= pseudobasal process) may have assisted the change in relations of the nerves and their foramina.

11. Summary.

1. The chondrocranium and the mandibular arch have been studied in seven stages. A summary of the changes in the mandibular arch during metamorphosis is given on p. 517 et seq.

2. These changes are discussed on p. 522 et seq.

3. A summary of the homologies which can be established or suggested by a study of the stages is given on p. 531. There are four new suggestions.

4. The temporal region of the skulls of Ascaphus, Liopelma, and Alytes are redescribed on p. 531, and are discussed on p. 538.

5. Ascaphus is shown to possess (in the adult) separate trigeminal and facial foramina divided by a prefacial commissure; a post-palatine commissure; with less certainty, a complete basitrabecular process articulating with a true basal process; and a trigeminal foramen divided possibly by the root of the ascending process.

6. Liopelma is shown to possess the above structures except for the divided trigeminal foramen, with the difference that the root of the basitrabecular process has been lost.

7. Alytes, Bombinator, and Discoglossus each possess a prefacial commissure. In them, and in all the other Anura, the isolated outer end of the basitrabecular process is probably represented as the pseudobasal process (Gaupp's basal process).

The structures described in sections 5, 6, and 7 of this summary have not previously been given these homologies.

8. The change from the Ascaphus to the Ranid type of temporal arrangement is shown to be due to a collecting together of the branches of nerves V and VII into a single pro-otic foramen.

9. Kruijtzer's theory of the hyoid suspension of the mandibular arch is criticized.

10. An interpretation is given of the probable evolution of the tadpole mandibular apparatus, and of the metamorphic changes required to produce the adult skull.

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13. Abbreviations used in the Plates.

ac., auditory capsule; *ajc.*, anterior jaw cartilage (cartilago labialis inferior, Gaupp); *an.*, articular notch for lower jaw; *asc.*, anterior semicircular canal; *bpr.*, basal process (of quadrate); *bt.*, basitrabecular process; *ca.*, columella auris (= plectrum); *cal.*, cartilago alaris (nasi); *cf.*, cranial floor; *ch.*, ceratohyal (cornu hyale); *ci.*, crista intermedia (nasi); *cls.*, supra-rostral cartilage (cartilago labialis superior, Gaupp); *co.*, cartilago obliqua (nasi); *cp.*, crista parotica; *cpi.*, cartilago prœnasalis inferior; *cps.*, cartilago prœnasalis superior; *cq.*, cranio-quadrate passage; *cqa.*, commissura quadrato-cranialis anterior; *ct.*, cornu trabeculae; *fa.*, foramen apicale, V_1; *faa.*, foramen acusticum anterius; *fc.*, foramen cranio palatinum; *fcp.*, foramen caroticum primarium; *fen.*, foramen endolymphaticum; *ff.*, facial foramen; *fj.*, foramen jugulare; *focn.*, foramen oculomotorii; *fol.*, foramen olfactorium; *fon.*, foramen opticum; *forn.*, foramen orbitonasalis, V_1; *fp.*, foramen pro-oticum; *fpi.*, foramen perilymphaticum inferius; *ft.*, foramen trigemini; *ftd.*, foramen trigemini (dorsal division, V_2 and V_3); *ftrn.*, foramen for IV; *ftv.*, foramen trigemini (ventral division, V_1); *gp.*, ganglion pro-oticum; *ha.*, attachment for hyoid cornu; *lac.*, 'cartilage ledge of auditory capsule'; *lc.*, lateral semicircular canal; *lj.*, lower jaw; *lq.*, ligamentum quadrato-ethmoidale; *lw.*, lateral cranial wall; *n.*, notochord; *nc.*, notch for articulation of ceratohyal; *nq.*, notch for quadrate; *op.*, operculum; *pa.*, processus ascendens (quadrati); *pan.*, processus antorbitalis; *paq.*, pars articularis (quadrati); *pf.*, foramen for VII palatine; *pfc.*, prefacial commissure; *pft.*, posterior fontanelle; *pjc.*, posterior jaw cartilage (cartilago Meckelii, Gaupp); *pma.*, processus maxillaris anterior; *pmp.*, processus maxillaris posterior; *pmq.*, processus muscularis (quadrati); *pn.*, paries nasi; *poa.*, processus oticus (adult); *pot.*, processus oticus (tadpole); *ppn.*, pars plana (nasi); *ppq.*, processus pseudopterygoideus (quadrati?); *pqe.*, processus quadrato-ethmoidalis (quadrati); *psc.*, posterior semicircular canal; *psp.*, 'pseudobasal process'; *psq.*, posterior spur of the quadrate; *ptc.*, processus pterygoideus (quadrati); *q.*, quadrate; *qd.*, quadrate debris; *sn.*, septum nasi; *son.*, solum nasi; *sst.*, subocular shelf of trabecula; *t.*, trabecula cranii; *tb.*, bridge dividing trigeminal foramen; *tm.*, taenia tecti marginalis; *tme.*, taenia tecti medialis; *tn.*, tectum nasi; *tql.*, 'trabecular-quadrate ligament'; *ts.*, tectum synoticum; *tt.*, taenia tecti transversalis; *Vd.*, 'dorsal nerve' of V (Wagner);

Vg., Trigeminal ganglion; *Vr.*, root of V; *Vv.*, 'ventral root' of V (Wagner), V_2 and V_3?; *V_1*, profundus nerve; *V_2*, maxillary branch of V; *V_3*, mandibular branch of V; *VIIct.*, mandibularis internus of VII (= chorda tympani); *VIIg.*, facial ganglion; *VIIhm.*, hyomandibular branch of VII; *VIIp.*, palatine branch of VII; *IXg.*, IX (glossopharangeal) ganglion; *IXd.*, dorsal branch of IX; *IXrc.*, ramus communicans IX to VII.

14. DESCRIPTION OF PLATES 33–45.

Figs. 1–25, and fig. 31, of R a n a   t e m p o r a r i a .

PLATE 33.

Fig. 1.—Stage 1. Lateral view.

Fig. 2.—Stage 2. Lateral view.

PLATE 34.

Fig. 3.—Stage 3. Lateral view.

Fig. 4.—Stage 4. Lateral view.

PLATE 35.

Fig. 5.—Stage 5. Lateral view.

Fig. 6.—Stage 6. Lateral view.

PLATE 36.

Fig. 7.—Stage 7. Lateral view.

Fig. 8.—Stage 1. As fig. 1, but with part of quadrate bar and auditory capsule cut away; the jaw cartilages are not figured.

PLATE 37.

Fig. 9.—Stage 2. As fig. 2, but with part of quadrate bar, auditory and nasal capsules and lower jaw removed.

Fig. 10.—Stage 3. As fig. 3, but with part of quadrate bar, auditory and nasal capsules and lower jaw removed.

Fig. 11.—Stage 4. As fig. 4, but with part of quadrate bar and part of auditory and nasal capsules and the lower jaw removed.

Fig. 12.—Stage 5. As part of fig. 5; lateral part of quadrate and auditory capsule removed.

PLATE 38.

Fig. 13.—Stage 4. Anterior view of posterior $\frac{2}{3}$ of the skull and jaws of the right side; the cut passes vertically through fig. 4 just in front of the tip of the commissura quadrato-cranialis anterior.

Fig. 14.—Stage 5. Anterior view of right side of the skull and jaws. The cut passes vertically through fig. 5, in the region of the optic foramen.

Fig. 15.—Stage 6. Anterior view of right half of skull and jaws. The cut passes vertically through fig. 6, between the optic and oculomotor foramina.

Fig. 16.—Stage 7. Anterior view of right half of skull and jaws. The cut passes vertically through fig. 7, between the optic and oculomotor foramina.

PLATE 39.

Fig. 17.—Stage 2. Dorsal view of left half of skull; lower jaw moved forward.

PLATE 40.

Fig. 18.—Stage 2. Dorsal view of left half of skull; lower jaw moved forward.

PLATE 41.

Fig. 19.—Stage 3. Dorsal view of left half of skull; lower jaw moved forward.

Fig. 20.—Stage 2. Lateral view of 'pseudobasal process'; quadrate and part of auditory capsule removed.

PLATE 42.

Fig. 21.—Stage 4. Dorsal view of left half of the skull; lower jaw moved forward and to the left.

PLATE 43.

Fig. 22.—Stage 5. Dorsal view of left half of skull; lower jaw moved forward and to the left.

Fig. 23.—Stage 5. Lateral view of 'pseudobasal process'; quadrate and part of auditory capsule removed.

Fig. 24.—Stage 6. Lateral view of pseudobasal process to which is fused quadrate debris; quadrate removed and part of auditory capsule cut away.

PLATE 44.

Fig. 25.—Stage 7.—Dorsal view of left side of skull; lower jaw moved forward and to the left.

PLATE 45.

Fig. 26.—Ascaphus truei, adult. Reconstruction of a thick section of the right side of the skull and jaws; anterior view, reconstructed from the published figures of de Villiers (1934) with the addition of two sections taken more anteriorly. No differentiation has been made between cartilage and cartilage bone.

Fig. 27.—Ascaphus truei, adult. As fig. 26, but with part of the palatoquadrate removed, and with nerves added.

Fig. 28.—Liopelma hochstetteri, adult. Reconstruction of a thick section of the right side of the skull and jaws; anterior view. Reconstructed from the combined published figures of Wagner (1934 i–1934 ii). No differentiation has been made between cartilage and cartilage bone.

Fig. 29.—Liopelma hochstetteri, adult. As fig. 28, but with part of the palatoquadrate removed and with nerves added.

Fig. 30.—Alytes obstetricans. Reconstruction of a thick section of the right side of the auditory capsule of a tadpole. From sections in fig. 3, a–h, van Seters (1922).

Fig. 31.—Rana temporaria, young adult. As fig. 20, but with nerves and hyoid cornu added.
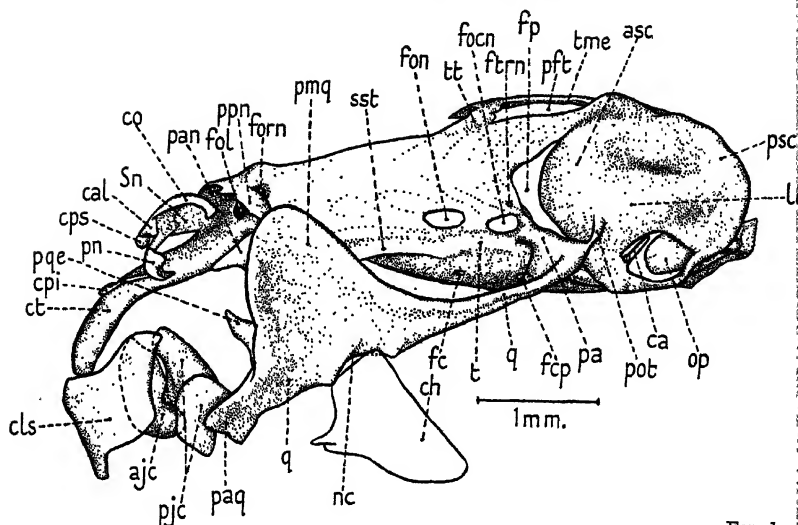
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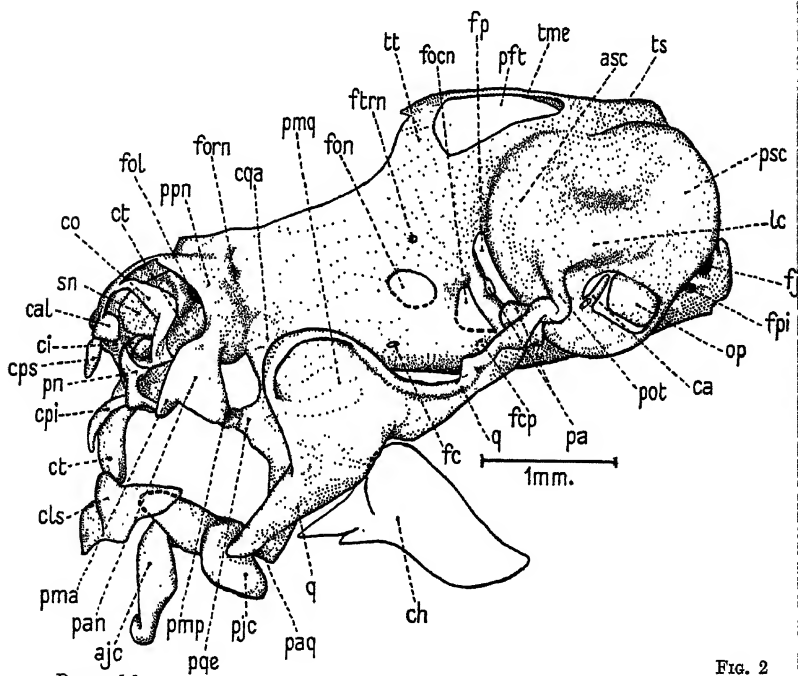
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FIG. 3

Pusey, del.

FIG. 4

Fig. 5

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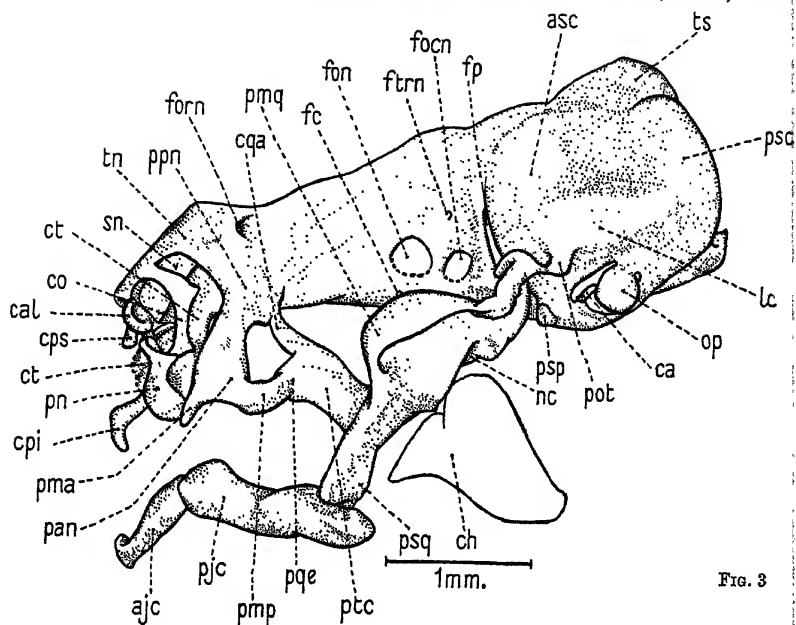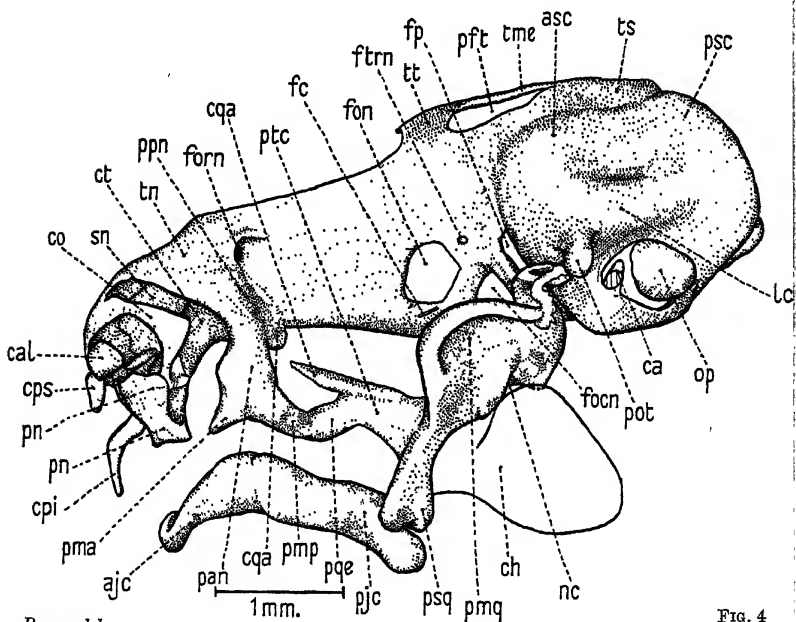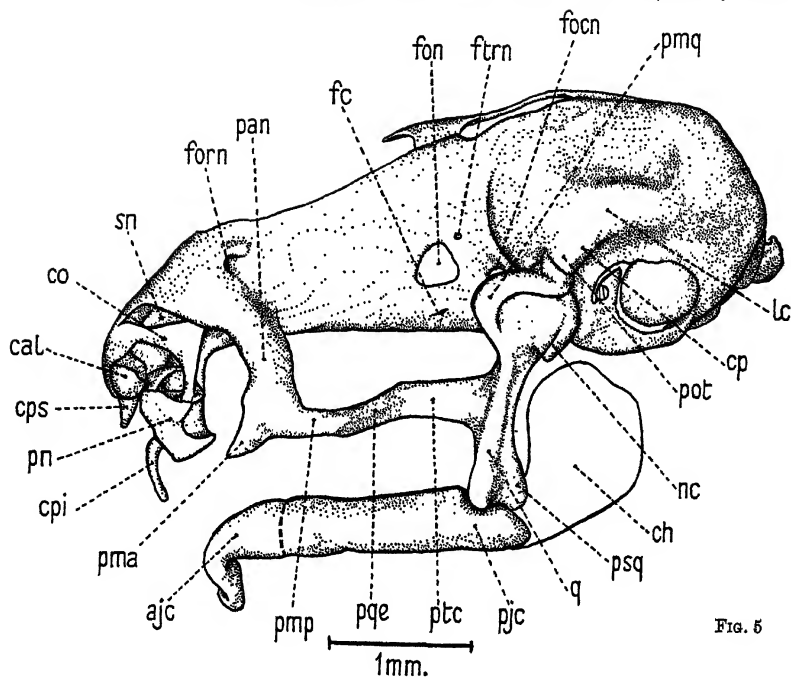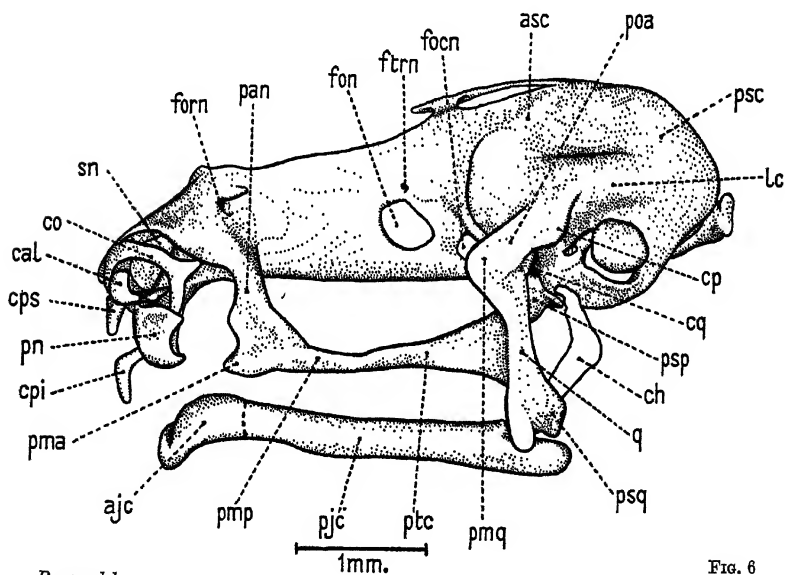
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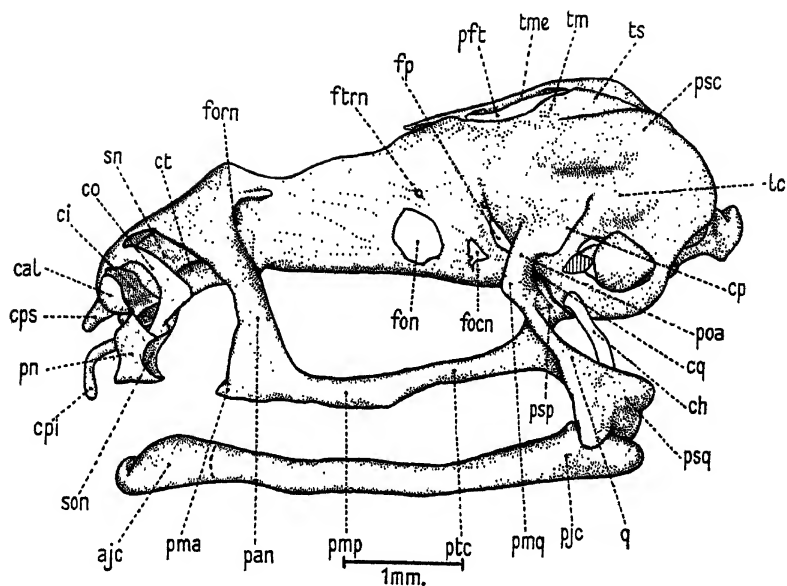
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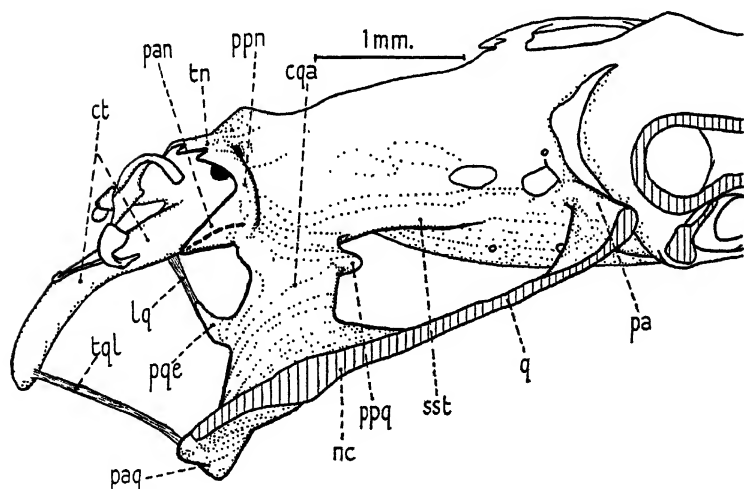
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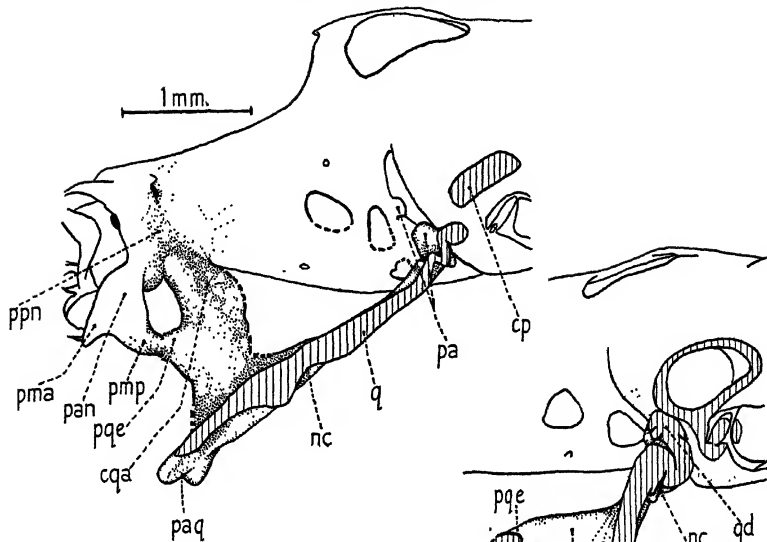
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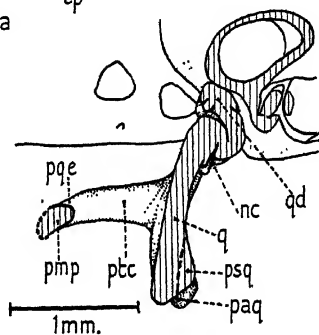
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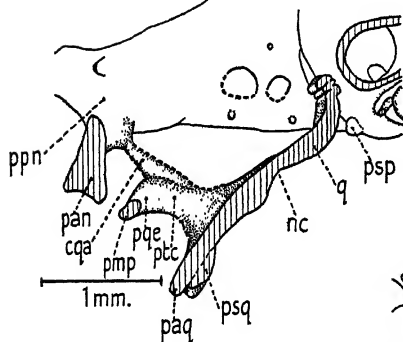
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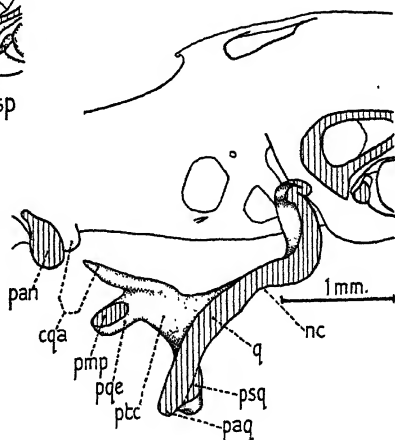
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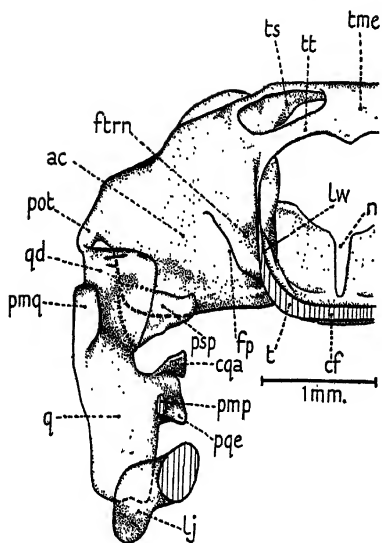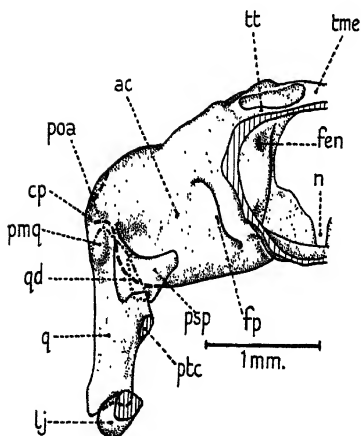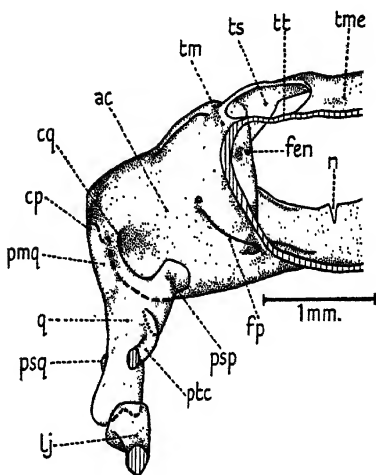
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FIG. 13

FIG. 14

FIG. 15

FIG. 16
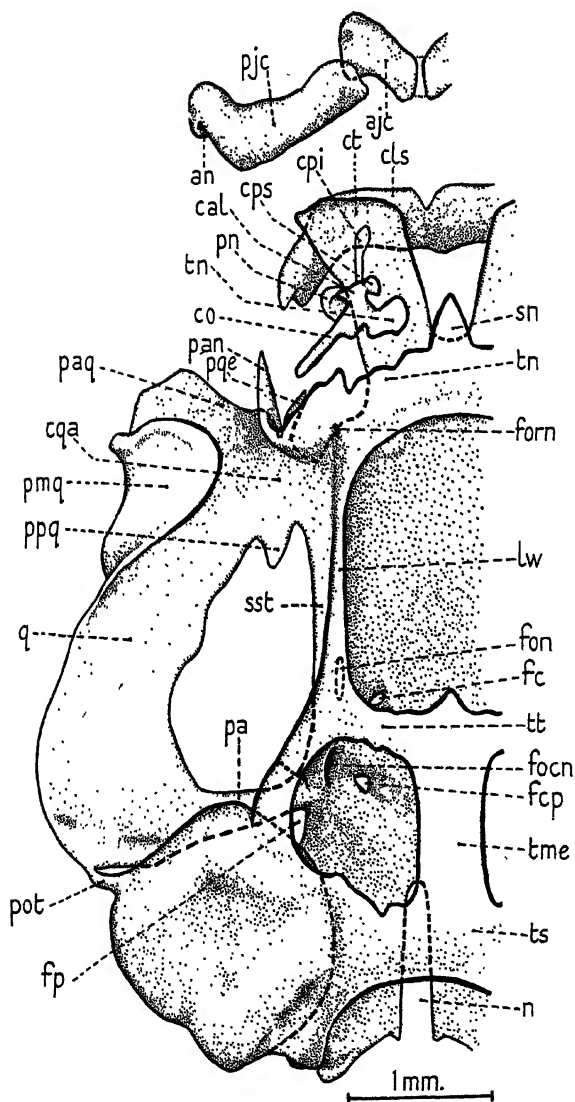
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FIG. 19

psp   FIG. 20

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Fɪɢ. 21

Fig. 22

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Fig. 23

Fig. 24

Pusey, del.

Fig. 25

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Fig. 26

Fig. 28

Fig. 27

Fig. 29

Fig. 30

Fig. 31
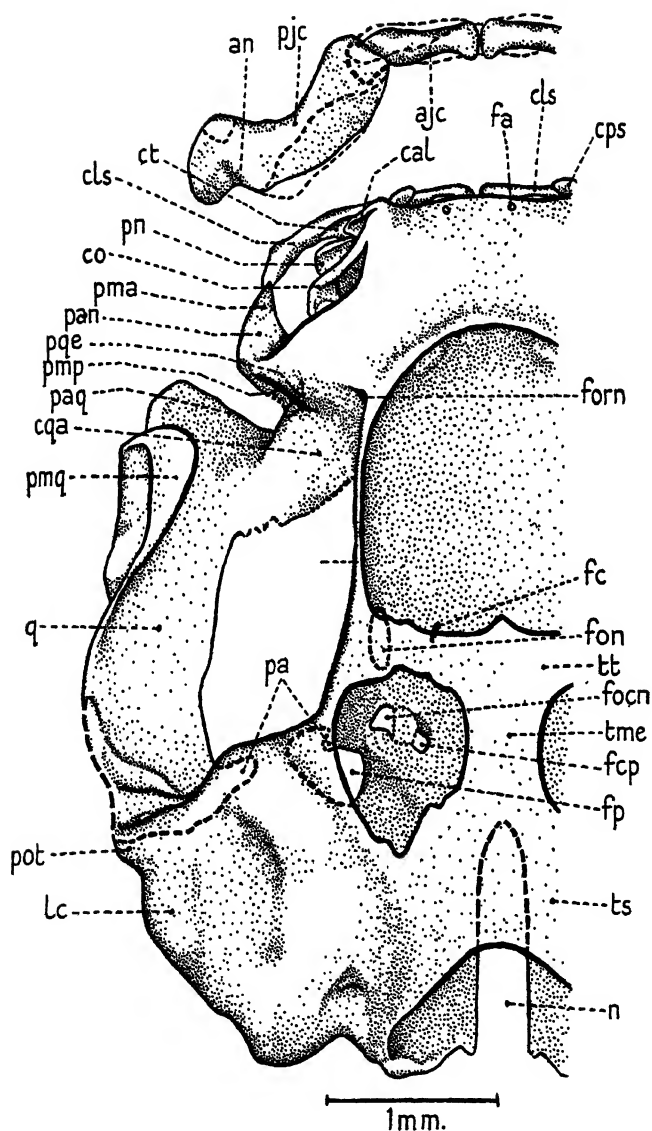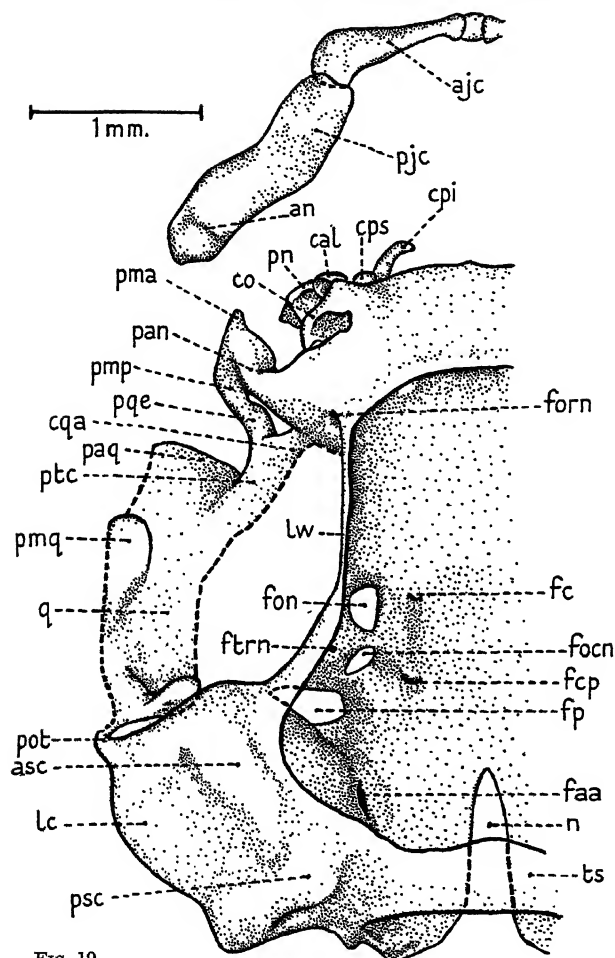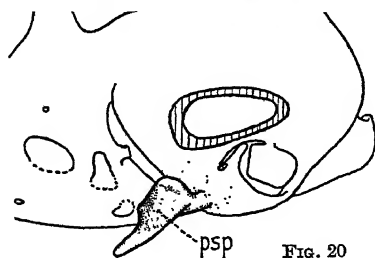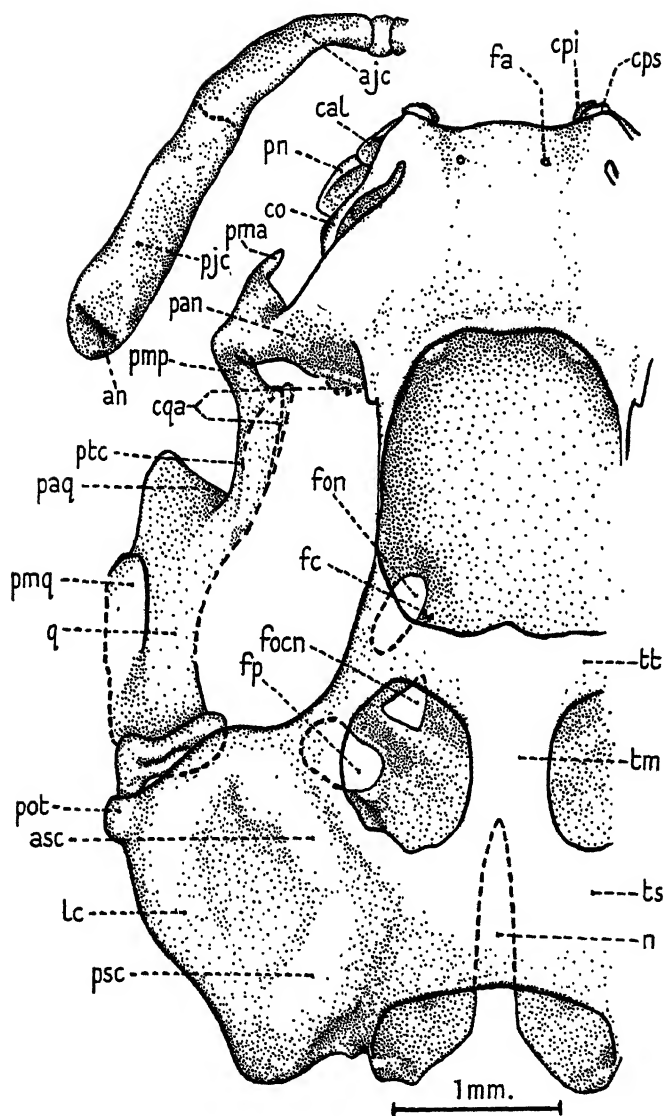
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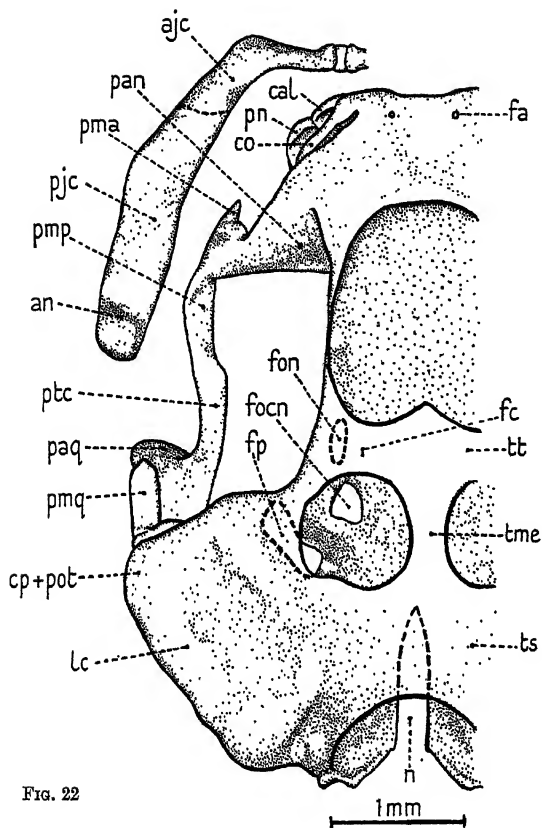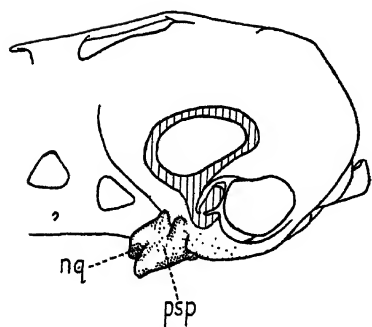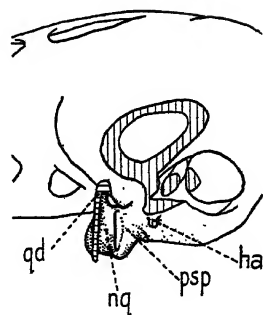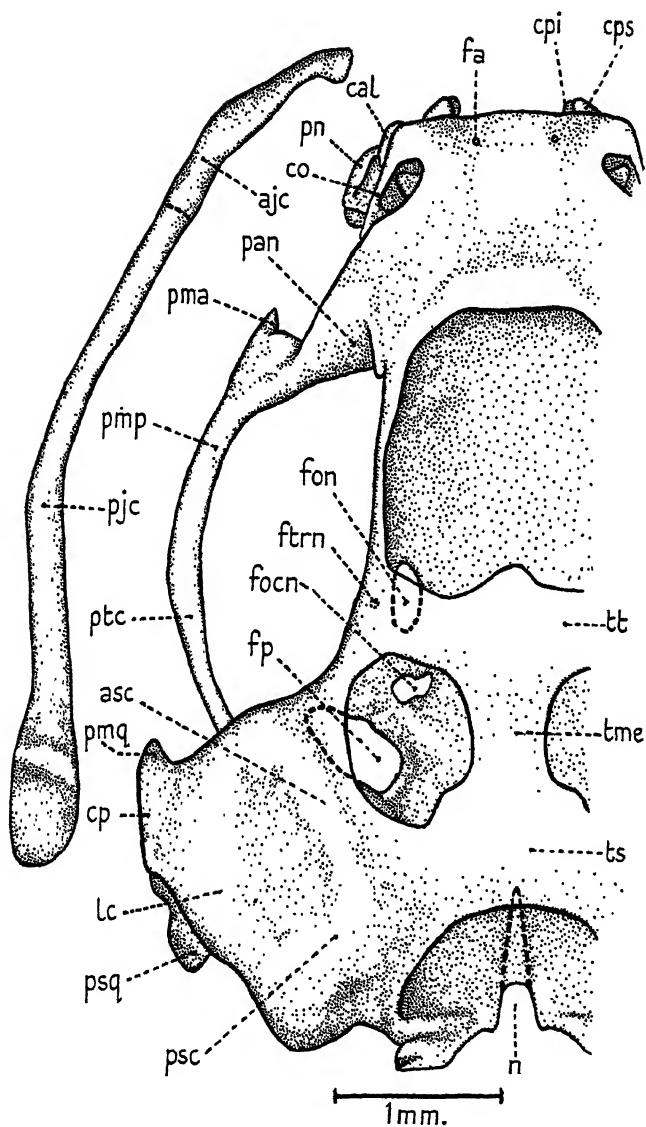
The Origin and Nature of the Egg Membranes in Chirocephalus diaphanus.

By

Mary L. Mawson, M.Sc., and C. M. Yonge, D.Sc.

University of Bristol.

With 8 Text-figures

Introduction.

The presence in the Anostraca of glands opening into the uterus was originally noted by Buchholz (1886) in Branchipus grubei. He suggested that they serve to secrete the egg-case, and later workers on Branchipus and on Artemia, notably Spangenberg (1875) and Claus (1886), accepted this view. Smith (1909), referring to Chirocephalus diaphanus, states that 'Short diverticula of the walls of the uterus receive the ducts of unicellular glands, the bodies of which contain a peculiar opaque secretion, said to form the egg-shells'.

The present research was designed to investigate the truth of this statement, and also to compare the nature and mode of formation of the egg-case in the Anostraca with that in the Decapoda. In the latter, as exemplified by Homarus, the egg-case consists of two membranes, an inner one of chitin secreted by the walls of the oviduct, and an outer one of cuticle secreted by the cement glands in the pleopods (Yonge, 1938).

Chirocephalus diaphanus Prevost was selected as the most suitable animal for this research. The majority of the specimens were collected on Dartmoor and were fixed in Bouin's fluid, which gave excellent results. Females at different stages of maturity were selected, the criterion of this being the length of the egg-sac. Serial sections were cut through those regions of the body containing the reproductive organs. Various combinations of stains were used, notably Mallory's triple stain, Delafield's or Heidenheim's haematoxylin in combination with

acid fuchsin, biebrick scarlet or eosin, and Mann's methyl blue eosin. Comparisons were then made between the condition of the glands and of the reproductive organs at different stages in the elaboration of the ovarian egg and its later passage into the egg-sac.

Living specimens were also obtained through the kind offices of Mr. A. G. Lowndes of Marlborough. By a fortunate chance, when two of these were fixed in Bouin's fluid eggs were observed to pass from the oviduct into the egg-sac. The condition of these eggs provided evidence of great value.

Finally, simple chemical tests were carried out on the egg-case to determine its composition.

ANATOMY OF THE FEMALE REPRODUCTIVE SYSTEM.

The female reproductive system (Text-figs. 1 and 2) consists of a pair of long tube-like ovaries (o.) which extend down the sides of the abdomen as far as the sixth abdominal segment. Towards their anterior end they tend to approximate on the ventral side. The oviducts (od.) appear as anterior prolongations of the ovaries, there being no definite line of demarcation between the two (see Text-fig. 3). In the region of the twelfth thoracic segment, the oviducts bend down sharply to open into a median unpaired uterus (u.) which is contained within the projecting egg-sac. In the very young individual the uterus is a simple elongated tube, but in the adult, two lateral pouches (l.p.) arise antero-ventrally and into these the oviducts open (Text-figs. 1, 2, and 3).

The uterine glands consist of two paired masses. The anterior pair (a.g.) are ventral, one on each side of the median line. The posterior and larger pair (p.g.) are situated one on each side of the body of the uterus extending round to the dorsal side, where they meet and extend forwards, in fully mature females, almost to the anterior limit of the anterior group. Each mass consists of a group of paired gland-cells with ducts communicating with the uterus. In the larger, more mature, individuals, the gland-masses are proportionally larger. This was found to be due not to an increase in number of the cells but to a notable enlargement of the individual gland-cells (compare Text-figs. 4 and 7).
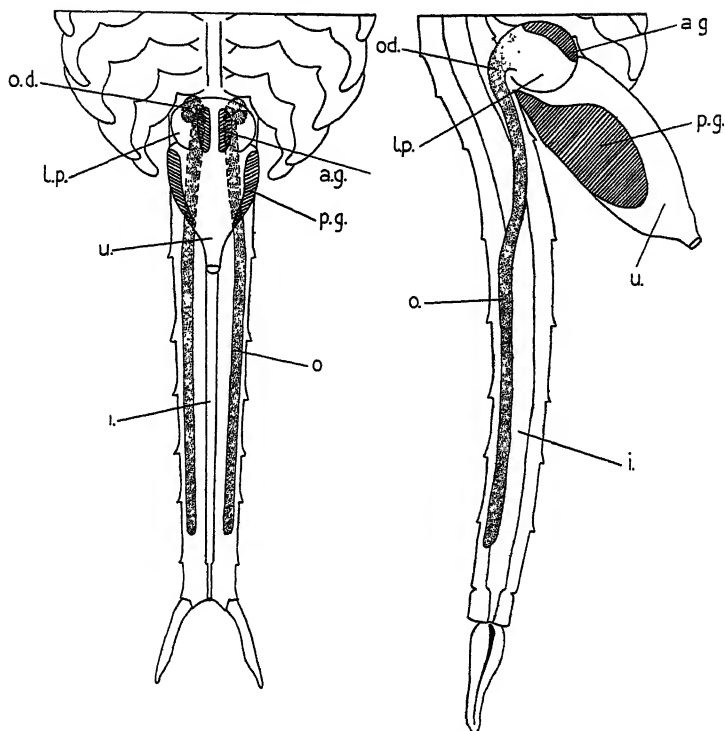
TEXT-FIG. 1.   TEXT-FIG. 2.

All the figures are of Chirocephalus diaphanus.
Fig. 1.—Ventral aspect showing the female reproductive organs.
×4. *a.g.*, anterior uterine glands; *i.*, intestine; *l.p.*, lateral pouch
of uterus; *o.*, ovary; *od.*, oviduct; *p.g.*, posterior uterine glands;
u., uterus.
Fig. 2.—Lateral aspect showing the female reproductive organs. ×4½.
Lettering as before.

THE UTERINE GLANDS.

The gland-cells may best be described in terms of their
development, details of which are given below. Like those of
Branchipus stagnalis and Branchipus torticornis,
described by Buchholz (1866) and by Claus (1886) respectively,
the gland-cells are arranged in pairs surrounded by a common
membrane. In Branchipus grubei, on the other hand,
Buchholz states that the gland-cells are separate, although a

single duct serves a pair of glands. The glands consist of cyto-
plasm, which is gradually replaced by granular secretion, and
of a characteristically large cup- or basin-shaped nucleus. The
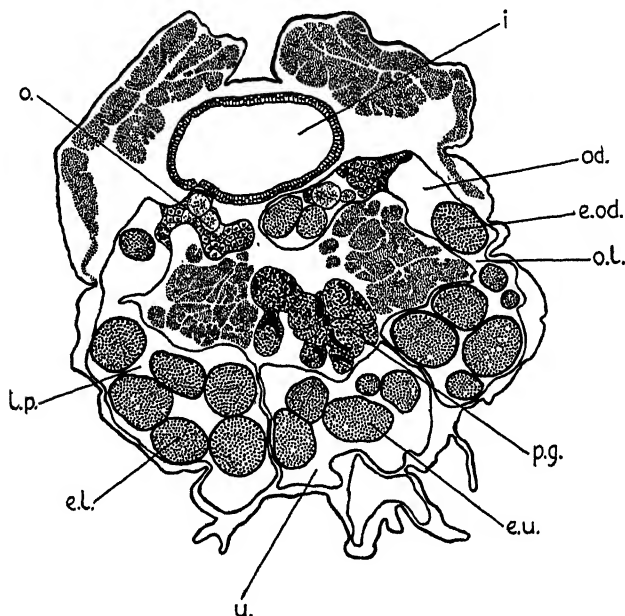
TEXT-FIG. 3.

Transverse section through a female at the junction between the ovi-
duct and the uterus. In this animal the stimulus of fixation caused
eggs to pass from the oviduct into the uterus, they do n o t there-
fore possess the rugose outer membrane. ×50. *e.l.*, eggs in lateral
pouch; *e.od.*, eggs in oviduct; *e.u.*, eggs in uterus; *o.l.*, opening of
oviduct into lateral pouch of uterus; *p.g.*, posterior uterine glands
full of secretion. Other lettering as before.

concavities of the nuclei of associated gland-cells face one
another. In the early stages of development the nuclei contain
large granules which first increase and later decrease in number.
These granules, and the secretion which forms in the cytoplasm,
give similar staining reactions, red or orange, with Mallory's
triple stain, red with Mann's methyl blue eosin, red with Dela-
field's haematoxylin and with acid fuchsin, and black with iron
haematoxylin. It appears, therefore, that the secretion is

elaborated in the nucleus and passed into the cytoplasm. The cytoplasm and the smaller granules in the nucleus stain in the usual way with cytoplasmic and nuclear stains respectively.

The earliest stage in which the glands were studied was in animals which possessed no egg-sac and no yolk in the ovarian eggs. The uterine glands (Text-fig. 4) were small, the pairs being about 29μ in diameter. The nucleus (n.) had a granular
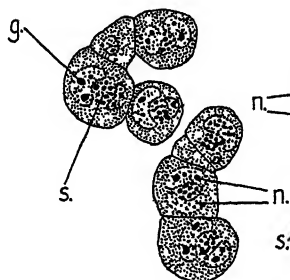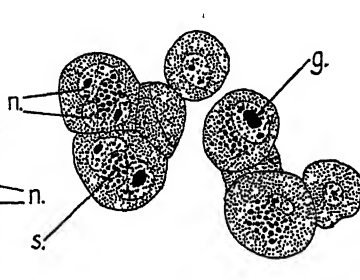
TEXT-FIG. 4.   TEXT-FIG. 5.

Fig. 4.—Section through uterine glands from female with no egg-sac and no yolk in the ovarian eggs. × 300. g., large granule in nucleus; n., paired nuclei; s., secretion in cytoplasm between paired nuclei.

Fig. 5.—Section through uterine glands from female with egg-sac 0·64 mm. long but with no yolk in the ovarian eggs. × 300. Lettering as before.

appearance, but the darkly staining bodies (g.) were not numerous. The secretion (s.) was confined to small masses lying chiefly within the cup of the nucleus. It consisted of granules somewhat smaller than the largest of those in the nucleus.

The next stage was found in animals with an egg-sac 0·64 mm. long and 0·18 mm. wide, yolk being still absent from the ovarian eggs. The glands (Text-fig. 5) were considerably larger, pairs averaging some 45μ in diameter. The size of the nucleus and the amount of secretion in the cytoplasm had increased proportionally. Irregular, vividly staining bodies were conspicuous in the nucleus, and the granules of secretion were larger as well as more abundant. The secretion was still mainly situated within the cups of the nuclei.

The third stage examined was in more mature animals where

the egg-sac was some 2·2 mm. long and 0·9 mm. wide. Here yolk was present in the ovarian eggs, and fully developed eggs were present in the oviduct and in the uterus. The pairs of glands (Text-fig. 6) had increased in diameter up to 80μ. The
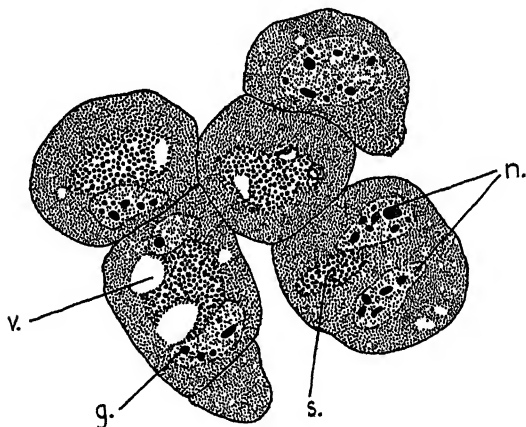
TEXT-FIG. 6.

Section through uterine glands from female with egg-sac 2·2 mm. long and with yolk in the ovarian eggs. ×300. *v.*, vacuole in cytoplasm. Other lettering as before.

granular, brightly staining, masses in the nucleus were more conspicuous and had increased in proportion to the general substance of the nucleus. The granules of secretion in the cytoplasm were slightly larger, and had increased in amount to a greater extent than had the nucleus. Occasional large vacuoles (*v.*) appeared within the secretion.

The last series of animals sectioned consisted of fully mature individuals with egg-sacs up to 3·2 mm. long and 1·35 mm. wide. As before, eggs were present in the oviduct and in the uterus (see Text-fig. 3). The paired gland-cells (Text-fig. 7) in these animals attained a diameter of 130μ, almost five times that of the glands in the earliest stage (compare Text-figs. 7 and 4). In these the nuclei (*n.g.*) broke down, becoming eventually no more than irregular masses of darkly staining material. The remainder of the cell became finally exclusively filled with secretion (*s.*),
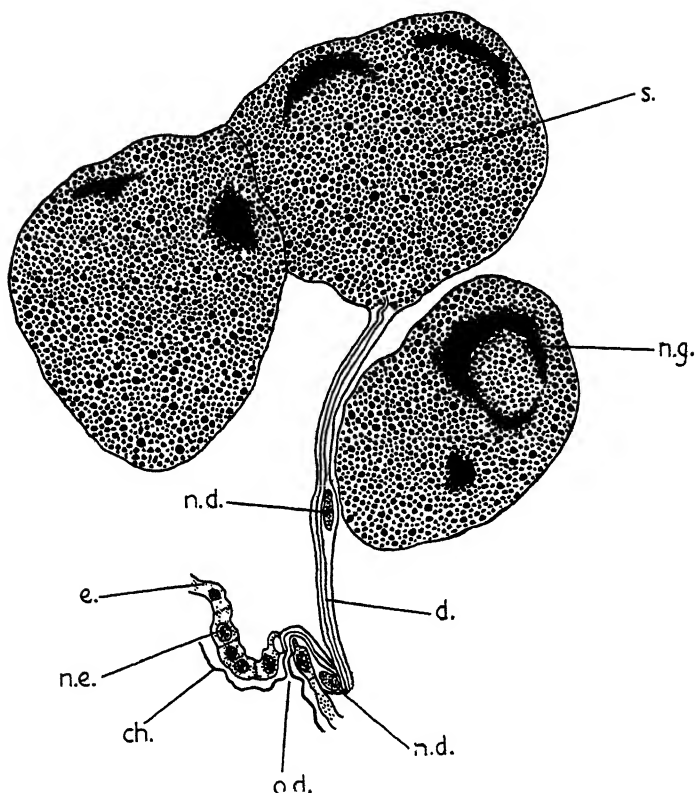
TEXT-FIG. 7.

Section through uterine glands of fully mature female with egg-
sac 3·2 mm. long and with eggs in the oviduct and uterus. × 300.
ch., chitinous intima of uterus; *d.*, duct of uterine gland; *e.*,
epithelium of uterus; *n.d.*, nucleus of duct (two are shown n o t
belonging to the same duct); *n.e.*, nucleus of uterine epithelium;
n.g., nucleus (degenerating) of gland-cell; *o.d.*, opening of duct into
uterus; *s.*, secretion filling gland-cell.

the granules of which were much larger than in earlier stages.
No trace of the original cytoplasm remained.

DUCTS OF THE UTERINE GLANDS.

The structure of the ducts in the fully developed glands is
shown in Text-fig. 7 (*d.*). They consist of long, very narrow

tubes extending from the wall of the uterus to the paired gland-cells. Each duct is unbranched and communicates with a single pair of gland-cells. The lumen of the tube, which is very well defined, has a diameter about equal to the thickness of the walls. Each duct possesses a single oval nucleus (*n.d.*) which is situated in a small swelling in the wall of the duct. Except for their oval shape, conditioned by the nature of the duct, they are very similar in appearance to the nuclei of the uterine epithelium (*n.e.*), possessing usually a single nucleolus and many small granules. This epithelium secretes a thin integument (*ch.*), presumably of chitin, and this appears to pass into the lumen of the ducts. If such is the case it would explain the sharp definition of the lumen. In this connexion it is interesting to note that Kinzig (1914) believed that the ducts of the tegumental glands which line the statocyst of Decapoda are bounded with chitin; this has also been the impression of one of us (C. M. Y.) in the case of the tegumental glands of the oesophagus and labrum in Homarus.

These similarities between the ducts and the uterine epithelium suggest that the former may represent ingrowths from the epithelium to the glands. Careful study of sections of the earliest stage available in the elaboration of the glands revealed that ducts are present though clearly not functional and still in process of elaboration. The epithelium of the uterus is very irregular at this stage, indicating a possible inward growth of the cells which are forming the ducts. The nuclei of these are much larger, at this stage, than those of the epithelium, but this may be due to their great activity. There is no definite evidence that the ducts are n o t growing inwards; on the other hand, this cannot be regarded as proved.

NATURE AND FORMATION OF THE EGG-CASE.

The structure of the egg-case is shown in Text-fig. 8. The yolky egg-mass (*y.*) is surrounded by a thin inner membrane (*i.*) which adheres closely to the egg-mass even after sectioning. Surrounding this is the thicker, rugose outer membrane (*o.*) which gives the characteristic spiny appearance to the eggs of

Chirocephalus. This membrane tends to come away from the inner one in the process of sectioning.

Neither of these membranes is present around the ovarian egg. Sections of mature females with eggs in the oviduct and in the uterus reveal that the former possess the inner, smooth membrane only, whereas the spiny outer membrane is also present around those in the uterus. This indicates that the inner
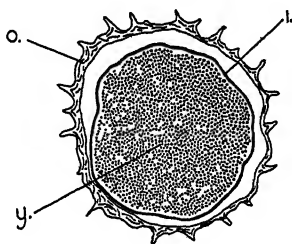
TEXT-FIG. 8.

Section through uterine egg. ×150. *i.*, inner, chitinous membrane; *o.*, outer, rugose, non-chitinous membrane; *y.*, yolk.

membrane is formed by the oviduct and the outer one by the uterine glands. Fortunate confirmation of this was obtained from sections of animals in which the act of fixation had brought about the passage of eggs into the uterus. Text-fig. 3 represents a section through the reproductive region of one of these animals. It will be seen that in this case not only are the eggs in the oviduct (*e.od.*) devoid of the outer membrane but also those in the uterus (*e.u.*) and in the lateral pouches of this (*e.l.*). Apparently, therefore, fixation brought about contraction of the oviduct, causing expulsion of eggs already coated with secretion from the oviducal epithelium into the uterus; but the uterine glands were unable to discharge their secretion, so that the eggs failed to possess the outer membrane which in all other cases surrounded the eggs in the uterus. This view was confirmed by the condition of the uterine glands (*p.g.*) which were filled with secretion.

The structure of the epithelium of the oviduct is indistinguishable from that of the uterus, consisting of flattened cells,

the boundaries of which are difficult to determine in sections, and with somewhat irregularly disposed rounded or oval nuclei. But, whereas a very thin chitinous integument can be seen in favourable preparations of the uterus (see Text-fig. 7, *ch*.), this appears to be absent in the oviduct.

Chemical examination of the egg-membranes provided further evidence of value. Eggs possessing both membranes were tested for chitin by the method of Campbell (1928). After the initial boiling in NaOH in a glycerine bath at 160° C. for 15 minutes the outer membrane was dissolved, indicating that it is not chitinous, but the inner membrane remained. When this was treated with iodine followed by sulphuric acid the deep mauve colour characteristic of chitosan was obtained. The inner membrane is therefore chitinous. Eggs were also subjected to the action of concentrated hydrochloric acid for one day, and the outer membrane remained intact. This provided further evidence that it is non-chitinous and also indicated possible similarity to the superficial cuticle of the integument of Homarus (Yonge, 1932) which also forms the outer membrane around the eggs of Decapoda (Yonge, 1938).

Discussion.

It is interesting to find that the egg-membranes in Chirocephalus diaphanus are of the same dual character as those of Homarus vulgaris, and with little doubt of all other Decapoda which attach the eggs to the pleopods (Yonge, 1938). In both the inner membrane is chitinous and is secreted by the walls of the oviduct. The outer membrane in the case of Chirocephalus possesses at least one of the properties of the cuticle which form the outer egg-membrane in Homarus (Yonge, 1932, 1938), namely resistance to attack by concentrated hydrochloric acid, and, from its mode of formation, probably also that of low surface tension. The presence of two egg-membranes has also been recorded by Ziegelmayer (1926) for Copepoda.

When discussing the significance of these two membranes in Homarus (Yonge, 1938) it was pointed out that egg-laying is associated with ecdysis, occurring at some definite period after

this, and involving the same two processes of secretion: formation of underlying chitin by an epithelium (of the ectodermal surface generally or of the oviduct), and of a superficial cuticle by glands (tegumental in the one case, cement in the other, the two being structurally indistinguishable). Certainly in the Decapoda the cement glands secrete a substance indistinguishable chemically or physically from that produced by the tegumental glands. The properties of the cuticle secreted by the latter—low surface tension, slow solidification in water, final hardness, and limited permeability—are exploited to provide an outer egg-membrane which is much harder than chitin, provides an attachment to the pleopods, and, by its strictly limited permeability (Yonge, 1936), provides not only a greater insulation of the developing egg from changes in the environment, but also the possibility of osmatic hatching (see Needham, 1931, pp. 1600–2, for resumé of literature on this subject).

In Chirocephalus the outer egg-case is purely protective in character, its substance being not prolonged into a 'funicula' providing attachment to the female. Protection is of supreme importance in a species the eggs of which must resist prolonged desiccation and, presumably in correlation with this, the outer membrane is relatively much thicker than it is in the Decapoda. The cement glands of the Decapoda being modified tegumental glands, there appears some reason for regarding the uterine glands of the Anostraca as homologous with the very numerous body and leg glands. Spangenberg (1875) came to this opinion on purely morphological grounds, but Claus (1886) strongly opposed this view, stating that not only was there difference in details of structure, but that, whereas the uterine glands are formed by inpouching of the uterus, the abdominal and leg glands originate as ectodermal structures. The former point appears of minor importance, while the latter depends on the truth of the statement that the uterus is mesodermal in origin, which, if the possession of chitinous intima be regarded as evidence of an ectodermal epithelium, can reasonably be disputed. A study of the leg and body glands of Chirocephalus lay outside the range of this research, but it was observed that their structure was of the same general character as that of the

uterine glands. Other work carried out in this Department, and briefly referred to elsewhere (Nicholson and Yonge, 1935), indicates that the leg and body glands may be concerned with cuticle formation, and, in view of the conditions prevailing in the Decapoda, the fact that the uterine glands certainly secrete a cuticle- or cement-like substance lends support to this view. It appears probable that the conditions in the Anostraca and in the Decapoda are essentially the same.

Summary.

1. The anatomy of the female reproductive system in Chirocephalus diaphanus is described with especial reference to the paired anterior and posterior masses of uterine glands.

2. The gland-cells are arranged in pairs surrounded by a common membrane and served by a single duct formed by a separate duct-cell, representing possibly an ingrowth of the uterine epithelium.

3. The glands increase greatly in size during development owing to the formation of a granular secretion which is apparently formed in the nucleus and gradually displaces the original cytoplasm.

4. The egg-case consists of an inner membrane which is chitinous and is formed by the oviducal epithelium, and an outer thicker, rugose membrane which is non-chitinous and is secreted by the uterine glands.

5. Attention is drawn to the close resemblance to conditions in the Decapoda. The inner membrane in both cases is chitinous and formed in the oviduct, while the uterine secretion has much in common with the secretion of the cement glands, but is concerned with protection only and not also with attachment to the body of the female.

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The Golgi Apparatus of Copromonas subtilis, and Euglena sp.

By

J. Brontë Gatenby and B. N. Singh,

Zoology Laboratory, Trinity College, Dublin.

With Plates 46–48 and 9 Text-figures.

INTRODUCTION.

THE problems surrounding the morphology of the cytoplasmic inclusions of the germ-cells and gland-cells have largely been solved in recent years. The discovery of a Golgi apparatus in Sporozoa (Hirschler, 1914) and the papers of Nassonov (1924, 1925) on the possible connexion between Golgi material and contractile vacuole in various Protozoa have served, so far as our present work is concerned, to shift the search mainly to that part of the field associated with unicellular organisms. Recently Miss M. Daniels (1938) has successfully investigated the cytoplasmic inclusions of three species of Gregarina by means of the ultra-centrifuge. The present paper has arisen out of the work of Mrs. Lamont and one of the present authors (B. N. S.) on Nebela collaris and Amoeba proteus, respectively; in neither organism has a true Golgi apparatus been found, either by the use of the centrifuge, or by any recognized staining method. Our attention has therefore turned to the flagellates, on which Nassonov, Grassé, and Duboscq and others have already done interesting work in this particular field. We began by examining Copromonas, because we believed that it was one of the simplest monads known. Unfortunately we found that it was more complicated than the previous monograph of Dobell (1908) had led us to believe, and, because of some special problems which arose, we had to turn our attention to a larger organism which was better known. Euglena had already been studied in this laboratory by Miss Patten and Beams (1936), who had given a very satisfactory account of the

staining properties and relative specific gravities of the various granules. We had the opportunity of going over most of the material which had been used for their work. According to Wenyon (1926), Euglena and Copromonas are allied genera, and we certainly obtained considerable light on the conditions in Copromonas by studying Euglena as well.

PREVIOUS WORK ON COPROMONAS.[1]

The genus Copromonas was established by Dobell (1908). Later on several species of this genus were described by various workers.

According to Dobell's description, Copromonas subtilis is a simple monad provided with a single flagellum arising from a depression at the anterior end. This depression is the cytostome, which leads into a longitudinal tube, the cytopharynx. The flagellum runs along the wall of the cytopharynx for a short distance and originates from a basal granule. The base of the flagellum is usually associated with the reservoir, and at times the basal granule seems to be situated on the posterior part of the reservoir. There are one or two small contractile vacuoles which discharge their fluid into the reservoir. The latter does not pulsate, but sometimes it is absent, and thus it may be that it periodically collapses, driving out its contents.

In the nucleus is a central deeply staining mass surrounded by a clear zone which contains practically no chromatin. The nuclear membrane, which is achromatic in nature, is united to the central portion by achromatic strands. Dobell says, 'The nucleus lies somewhat posteriorly, and is not connected in any way with the flagellum, as is so often the case in flagellates. But I may call attention to the fact that in stained preparations a very distinct dark line is sometimes seen uniting the base of the flagellum to the nucleus. After examining a considerable

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[1] According to Wenyon (1926), Dobell's genus Copromonas is probably Stein's genus, Scytomonas. In 1878, Stein recognized a family, Scytomonadina, with a number of genera, Scytomonas, Petalomonas, Menoidium, Sphenomonas, etc. In his text, Dobell does not refer to Stein's genus. See, 'Der Organismus der Infusionsthiere', III Abt., page x, by F. R. von Stein, 1878.

number of monads which show this I am satisfied that it is
really due to the cytopharynx, the animal having rolled over
so that the cytopharynx appears to be in line with the flagellum,
and to connect it with the nucleus, over which the cytopharynx
has come to lie.'

In asexual multiplication by longitudinal division, the animal
becomes gradually motionless and the flagellum is completely
drawn in. During this process the nucleus becomes elongated.
Later on the basal granule divides into two, and from each of
these a new flagellum is developed. Meanwhile a cleft appears
between the bases of the flagella; this cleft, while extending
backwards, cuts the reservoir into two. Further extension of
the cleft divides the animal into two daughter individuals. One
contractile vacuole persists in one of the daughter individuals,
while a new one arises in the second.

Dobell has described the process of conjugation and encyst-
ment in Copromonas subtilis, giving a detailed account
of what happens to the nucleus during these phases of the life-
cycle. He was not able to shed much light on other structures
(basal granules, reservoir, contractile vacuoles, &c.). On con-
jugation, one reservoir apparently collapses and the other one
remains functioning. Sometimes both the reservoirs remain
functional up to a quite late stage. Cysts, when they are first
liberated from the large intestine of a frog or toad, have no
reservoir, cytopharynx, or food-bodies.

Dobell believes that the Copromonas type of nucleus is
the most primitive type, and the Euglena type of nucleus
the most highly evolved. In the latter type a 'nucleolo-centro-
some' (Keuten) is present which is absent in the former type,
according to Dobell.

The description given by Wenyon, of the same species of
Copromonas cultured from the pig's faeces, differs in some
respects from that of Dobell. Wenyon has described an intra-
nuclear centrosome or central granule, which has a definite
function in connexion with the division of the nucleus. Accord-
ing to him, the flagellum runs a longer way inside the animal
than has been indicated by Dobell, before it ends in a blepharo-
plast. During the division of the animal by longitudinal fission

the two daughter centrosomes are connected by a fibre-like structure.

MATERIAL AND METHODS.

The material for the present investigation was obtained by making cultures of Copromonas subtilis from the faeces of frogs, by the method described by Grassi and Schewiakoff (1888). We used a few c.c. more egg albumen than was used by Dobell (1908). The culture solution consisted of 40 c.c. of egg albumen, 1 gram NaCl, and 200 c.c. of distilled water. This gave better results in making smear preparations. It is not possible to say definitely in how many days a really good culture can be obtained. Sometimes it took more than two weeks before a good yield of Copromonas could be got for our work, and in such cultures dividing, conjugating, and encysted forms could be seen.

The methods used were those that have been described in 'Microtomist's Vade Mecum' (1937 ed.) and 'Biological Laboratory Technique' (Gatenby, 1937). Both silver and osmic methods were tried, but it was found that Weigl osmic technique was most satisfactory in demonstrating osmiophilic material during different phases in the life-cycle of Copromonas. Silver preparations were not very satisfactory for showing Golgi bodies, as is generally the case in other Protozoa, but they showed mitochondria, axostyle, and rhizoplast quite clearly. Other fixatives were also used—Bouin, Schaudinn, Hermann, Champy, and Champy-Nassonov, &c.

Tests for fat (Sudan IV method), volutin, and glycogen were also used. Neutral red was used in various dilutions—1/10,000 to 1/30,000 in normal salt solution. The stains mostly used were iron alum haematoxylin, acid fuchsin and methyl green, thionin, gentian violet, neutral red acetic, and Mann's methyl blue eosin. For the demonstration of the Golgi apparatus animals were fixed and osmicated in tubes. The hanging drop or coverslip preparations were not very satisfactory.

EUGLENA SP.

The arrangement of the cytoplasmic bodies in Euglena, according to the views which have been taught for generations, is shown in Text-fig. 2, after Borradaile (1938). There is a gullet,

immediately below which lies a delicate vesicle, the reservoir (R.), abutting against the wall of which is a second and smaller vesicle, the contractile vacuole (C.V.), which discharges periodically into the reservoir, which itself discharges at longer periods into the gullet. Around the contractile vacuole are accessory contractile vacuoles (A.V.) which surround the main vacuole and re-form it. These parts can all be seen in the living organism.

In 1931, André Sigot of Strasbourg demonstrated what he called 'plaquettes osmiophiles autour du reservoir', as shown in Text-fig. 1. Sigot, who is a follower of the late Dr. Parat's nomenclature, describes a 'vacuome' in the form of small neutral red stainable bodies, which, according to Miss R. Patten and H. W. Beams, are probably the volutin granules of other protozoologists. These granules are marked v in Text-fig. 1. Miss Patten and Beams recognize plastids, paramylum, and mitochondria as well. The manner in which all these bodies become layered on ultra-centrifuging is shown in Text-fig. 3, mitochondria (M.) being the heaviest, volutin and paramylum the lightest, the chloroplasts (C.P.) coming in between.

Miss Patten's material, which had been deposited in this Department, has been re-examined by the senior author. In figs. 1 and 2, Pl. 46, are two examples of Euglena prepared by the Weigl (Mann-Kopsch) method and bleached. Similar organisms are shown in Text-fig. 1 of Miss Patten and Beams's paper (1936). We find, however, contrary to Miss Patten and Beams, that in many of the organisms there is an additional vesicle in front of what Sigot, and Miss Patten and Beams, have called the reservoir. More recently we have investigated further material of Euglena, and find that in the viridis type the osmiophil material lies at the lower end of the reservoir, where it forms a separate vacuole, whereas in the gracilis type the osmiophil material is intimately related to the whole wall of the reservoir. Sigot is therefore quite correct in his statement ; in Miss Patten's slides both viridis and gracilis types of vacuole systems can be found. This matter is further discussed in a forthcoming paper in "La Cellule."

In Miss Patten's material all sorts of conditions of the osmiophil accessory contractile vacuoles may be found, varying

from the appearance shown in Borradaile's figure (Text-fig. 2), where they are uniform in size and applied to the wall of the large contractile vacuole (shown partly in fig. 2, Pl. 46), to a
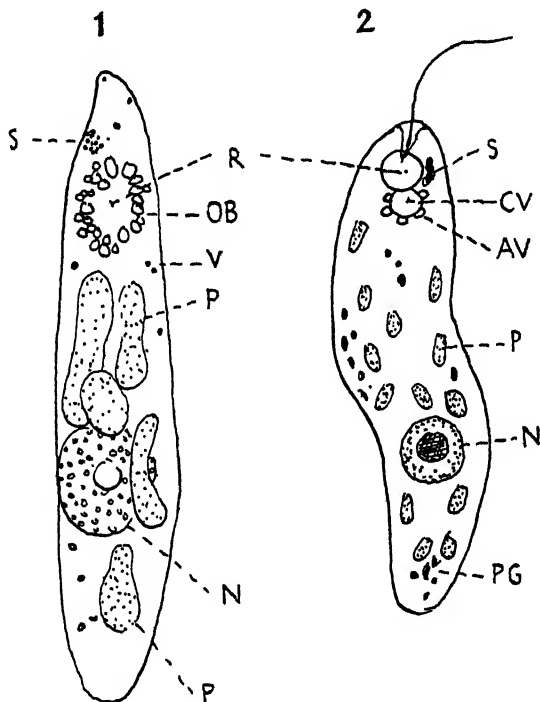
TEXT-FIGS. 1 and 2.

Fig. 1.—Euglena gracilis (after Sigot, 1931).
Fig. 2.—Euglena viridis (after Borradaile, 1938).
A.V., accessory contractile vacuoles; C., centriole; C.V., contractile vacuole; C.P., plastid layer; M., mitochondria; N., nucleus; N.R., neutral-red bodies; O.B., osmiophilic bodies; P., plastids; P.G. and P.M., paramylum; R., reservoir; S., stigma; V., 'vacuome'.

condition where this uniformity is interrupted by the swelling up of a few, or sometimes almost all of the accessory contractile vacuoles. These swollen bodies are well shown in the three figures in Pl. 46. Occasionally, as in fig. 1, Pl. 46, they almost completely embrace the contractile vesicle, but in the majority of cases the ventral side of the vesicle has attached to it a large

bladder-like structure (G.X.) in direct contact with the contractile vacuole. We believe that the body marked G.X., in figs. 2 and 3, Pl. 46, is swollen with water and is ready to discharge into the contractile vacuole. It is, as has been assumed
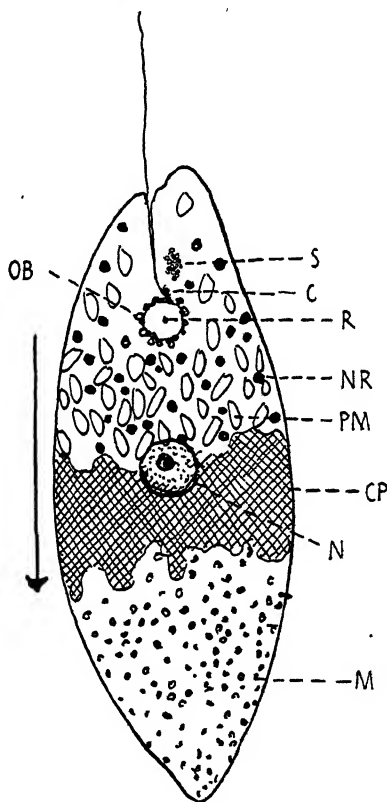
TEXT-FIG. 3.

Fig. 3.—Euglena sp. (after Miss Patten and Beams, 1936).

by previous workers who have carefully observed the living organisms, merely an accessory contractile vacuole. Probably in the enlarged condition it is more difficult to observe in the living state. We do not know for certain whether this is so.

It will be seen, therefore, that our conception of the contractile vacuole and reservoir apparatus of Euglena agrees in all

essentials with the conventional account found in the text-books. There is one point more we wish to emphasize—in the ultra-centrifuged Euglena the reservoir cannot be found immediately after centrifuging, and the contractile vacuole and its parts may be made to drift away from the anterior end of the organism, as shown in fig. 3, Pl. 46.

We now come to the question of the homology of the parts which are osmiophil. According to our view, the osmiophil substance represents the Golgi material of higher forms, and with the contractile vacuole constitutes a compound structure.

Copromonas.

In the photograph on fig. 16, Pl. 48, (G.) is a typical example of the osmiophil structure in Copromonas. Here is a very large, extremely osmiophil body, which, excepting the basal body of the flagellum, in nearly 90 per cent. of specimens is the only structure which becomes jet black after a few days' treatment in osmium tetroxide. Knowing Dobell's work on Copromonas, and bearing in mind the position and size of the reservoir of Copromonas, one naturally assumes at once that the osmiophil body is the blackened wall of the reservoir. This, however, is not the whole story. It is, indeed, true that in many cases the blackened vesicle is the only vacuole in this region of the organism, but in other cases a separate reservoir without osmiophil walls and abutting against the osmiophil vesicle may be seen, as, for example, in figs. 5 and 9, Pl. 46, and figs. 13–15, Pl. 47.

Now in the first place it must be mentioned that the amount and arrangement of the osmiophil substance may be very variable. Compare, for example, figs. 11 and 13, Pl. 47, with figs. 5 and 8, Pl. 46. In some specimens, as in fig. 7, Pl. 46, and fig. 10, Pl. 47, the osmiophil material may be scanty, while in all preparations monads can be found in which no blackening may have taken place. We have found examples in division in which one side contained osmiophil material, the other none whatever. We are certain, however, from our knowledge of the technique, that in the majority of cases, if not in all cases, where no blackening has taken place, the fault has been due to the fact that the

reduction of the osmium tetroxide has been prevented by the particular surroundings of the organism on the smear. For, where monads are osmicated in a tube, and not on a smear, the percentage of examples containing blackened vacuoles or granules approaches 100 per cent., whereas in thick smears many more individuals have failed to blacken. This matter is referred to below, p. 586. At this juncture we may answer a question which will possibly have presented itself to the mind of the reader—namely, what proof is there that the blackening is not an artefact? Firstly, in individuals kept in osmic solution in a hanging drop the blackening may be seen to be appearing in the small vesicles previously known to be contractile vacuoles or around previously identified reservoirs, and, secondly and more cogently, the blackened material undergoes definite division and sorting out during the division of the organism. See the photographs in figs. 16–19, Pl. 48, and the drawings in figs. 11, 13, 14, and 15, Pl. 47. Lastly, in ultra-centrifuged individuals the mitochondria pass centrifugally, whereas the lipoid or osmiophil material passes up centripetally against the cell-wall. It will therefore be unnecessary to labour the point further.

. It has been mentioned that a continually occurring form of osmiophil body is the structure shown in fig. 4, Pl. 46, and in fig. 16, Pl. 48. This perfectly spherical body is a phase of the cycle which we believe to be taking place throughout the life of the organism. In fig. 9, Pl. 46, is another phase. Here there is a distinct non-osmiophil reservoir (R.), partly embraced by a group of granules (G.), which are energetically osmiophil. In fig. 6, Pl. 46, is still another phase, in which there is a space or reservoir (R.) surrounded by blebbed structures strongly recalling the condition in Euglena (fig. 2, Pl. 46). Now in Chilomonas, Nassonov recognizes the spherical phase (diastole) and a granular[1] or collapsed phase (systole). We feel that the case of Chilomonas might bear further examination, though we recognize the possibility of such a simple condition.

Now Dobell describes from his observation of the living organisms a simple reservoir and one or two contractile vacuoles

[1] Presumably, as in Copromonas, the condition in which the osmiophil material divides during cell division in Chilomonas.

which are supposed by him to discharge periodically into the reservoir. Such a condition of affairs is shown presumably by our fig. 7, Pl. 46, where the reservoir (R.G.) has one satellite contractile vacuole (G.). Likewise in fig. 10, Pl. 47, there are two reservoirs and two contractile vacuoles. But our observations lead us to believe that this condition is not any commoner than the other types already mentioned, and again can only be regarded as one possible phase of the cycle.

From Nassonov's description of Chilomonas, we naturally began by looking for the type shown in fig. 4, Pl. 46, where there is a single osmiophil vacuole. For a time we believed that this was always the reservoir, and that where the granular condition existed as in fig. 9, Pl. 46, it represented a systolic phase. But the discovery of specimens such as figs. 5, 9, Pl. 46, 13, 14, and 15, Pl. 47, where a non-osmiophil reservoir (R.) could be quite clearly seen, showed that the matter was more complicated. Furthermore, such examples as that of fig. 5, Pl. 46, where two equal osmiophil spheres (G.) as well as a reservoir (R.) existed side by side just before the onset of division suggested that in such cases the osmiophil spheres were preparing for the division. Both of us have found many stages like those in figs. 5 and 8, Pl. 46, but they cannot be regarded as the only, or commonest, type of prophase of division.

DIVISION.

Before continuing with a description of the resting phases and endeavouring to interpret them, it will be advantageous to examine some division stages. In the photographs on Pl. 48, it will be seen that the osmiophil material in fig. 16, Pl. 48, leaves its position, and, becoming more irregular in outline, drifts down to the upper middle line of the dividing individual, fig. 17, Pl. 48, and splits into two parts, figs. 18 and 19, Pl. 48. Comparable stages are drawn on figs. 13, 11, 14, and 15, Pl. 47, in order of division. Fig. 19, Pl. 48, shows a phenomenon which we have often noted, namely, that when the demarcation between the two attached individuals reaches the region wherein lie the food vacuoles, the osmiophil material may break up or bodily drift quite far down into the middle of the cell. This is

also noteworthy in fig. 15, Pl. 47, where in the right-hand organism a large vesicle has drifted below the nucleus.

Now a stage intermediate between that in figs. 17 and 18, Pl. 48, is shown in fig. 11, Pl. 47. This is very common, and is undoubtedly the usual method. The stage before it, in which the two nuclei are still in the dumb-bell stage, is drawn in fig. 13, Pl. 47. Here we have two reservoirs, and the osmiophil granules in much the same position as in the photographed organism in fig. 17, Pl. 48. In figs. 12, 14, and 15, Pl. 47, later stages are shown, the nucleus having divided completely. In fig. 14, Pl. 47, one reservoir is in diastole, the other partially in systole, and in fig. 15, Pl. 47, the two reservoirs, comparatively very large, are in diastole. In fig. 12, Pl. 47, the two organisms contain a great deal of osmiophil material, as in the photographs on Pl. 48.

RELATIONSHIP AND HOMOLOGY OF RESERVOIR AND CONTRACTILE VACUOLE.

Having shown that the osmiophil material is divided into two parts between the daughter organisms, it is now necessary to examine the questions surrounding the homology of the osmiophil and apparently non-osmiophil vacuoles. Examination of figs. 5, 7, Pl. 46, 10, 14, and 15, Pl. 47, shows that the degree of osmiophility of the vacuoles may vary considerably. In fact, it is impossible sometimes, as in figs. 7 and 10, to say whether the single vacuole present is a reservoir or a swollen contractile vacuole. In figs. 14 and 15, Pl. 47, the structures marked G are presumably contractile vacuoles, for there is a clearly marked reservoir as well. Likewise in fig. 5, Pl. 46, the bodies marked G are contractile vacuoles and not reservoirs, because a true reservoir is present. This homology has puzzled us considerably, but we believe that swollen osmiophil vacuoles (contractile vacuoles as in figs. 5 and 8, Pl. 46) can take the place of the true reservoir; and actually, in some specimens which in the living state show no reservoir, the clear space which presently appears is not necessarily re-formed in the exact site of the old reservoir, but is an enlarged osmiophil vacuole taking its place. In fig. 15, Pl. 47, for example, it is possible that in the right-hand organism the old reservoir, on collapse, will be

replaced by the growing osmiophil vacuole (G). The degree of osmiophility can then be a function of the amount of stretching of the osmiophil wall. In Pl. 47 some of the osmiophil vacuoles which are possibly in process of forming new reservoirs are marked RG.

EXAMPLES IN DIVISION WITH APPARENTLY A BLACKENED
     RESERVOIR OR CONTRACTILE VACUOLE IN ONLY ONE OF
     THE DAUGHTER CELLS.

It has already been mentioned that in smears the osmication of the Golgi material may be, in some cases, markedly capricious. In monads osmicated in a tube the number of dividing individuals in which one daughter organism contains an osmiophil structure, the other organism none, is greatly reduced. Even so, allowing for the well-known fact that isolated cells such as leucocytes or protozoa may not osmicate as evenly as occurs in pieces of metazoan tissue treated with a favourable specimen of commercial osmium tetroxide, it seems certain that in a small percentage of cases no osmiophil material does exist in one daughter cell. This must be due either to non-division of the existing osmiophil material or to some change in the chemical nature of the osmiophil material in one of the individuals. Further than to remark that the conditions depicted in the photomicrographs on figs. 17–19, Pl. 48, are the usual ones, and that such division stages as are shown on Pls. 46 and 47 among figs. 4–15 hold for most cells examined, we cannot go at present.

NOTE ON BODIES OTHER THAN THE OSMIOPHIL MATERIAL.

Our observations on Copromonas subtilis revealed structures which were overlooked by Dobell (1908) and Wenyon (1926). There is a stiff rod of apparently proteid nature which runs from the basal granule to the posterior end of the animal. We have identified this as the axostyle, figs. 4, Pl. 46, and 13, Pl. 47, and various Text-figs. In addition to this there is a rhizoplast connecting the basal granule with the nucleus, fig. 8, Pl. 46. We were able to see this structure in stained silver and osmic preparations. In properly differentiated Schaudinn preparations, stained with iron alum haematoxylin, both rhizoplast

and axostyle are very clearly seen, and we have no doubt that these structures are quite distinct and separate from the cytopharynx. We do not believe the statement of Dobell (1908), as quoted in the paragraph dealing with previous work, that the cytopharynx sometimes seems to have a connexion with the nucleus, and that there is no connexion between the nucleus and the basal granule. Dobell actually figured the axostyle in his Pl. 4, figs. 1, 2, 16, &c.

In the early stages of division, by longitudinal fission, in Copromonas, the basal granule is the first structure to divide, Text-figs. 4 and 5, and the daughter granules are always connected by a fibre or thread-like structure. Text-fig. 4 is a much earlier stage in the division of this body than has been indicated by Wenyon (1926). At this stage the nucleus is slightly elongated. The rhizoplast and axostyle are not seen after the division of the basal granule into two. We believe that both these structures probably disappear or degenerate during the early stages of division. The flagellum persists, although it is withdrawn considerably during the division of the basal granule. The second flagellum is developed from the second basal granule. The two axostyles are visible in fairly late stages in the division, when the nucleus has nearly divided into two, fig. 13, Pl. 47.

There is a considerable amount of disagreement regarding the question of the axostyle[1] during the division of an individual. According to Wenyon (1907) and Kofoid and Swezy (1915), the axostyle splits longitudinally into two. Dobell (1909) and others hold the view that the axostyles arise from the paradesmose. Kuczynski (1914), Wenrich (1921), &c., do not agree with the former views, and claim that the old axostyle disappears and new ones are formed as outgrowths from the blepharoplasts. We, as a result of our investigation in Copromonas, agree with the last group of workers, although we are not very definite on this problem. We have come to this conclusion from the fact that we could not see the axostyle, even in properly stained and differentiated preparations, during the division of the basal

[1] See Wenyon's (1926) 'Protozoology', vols. 1 and 2, and Hirschler (1932), 'Zeits. für Zell. und mikros. Anat.', 15 B., 4 H.
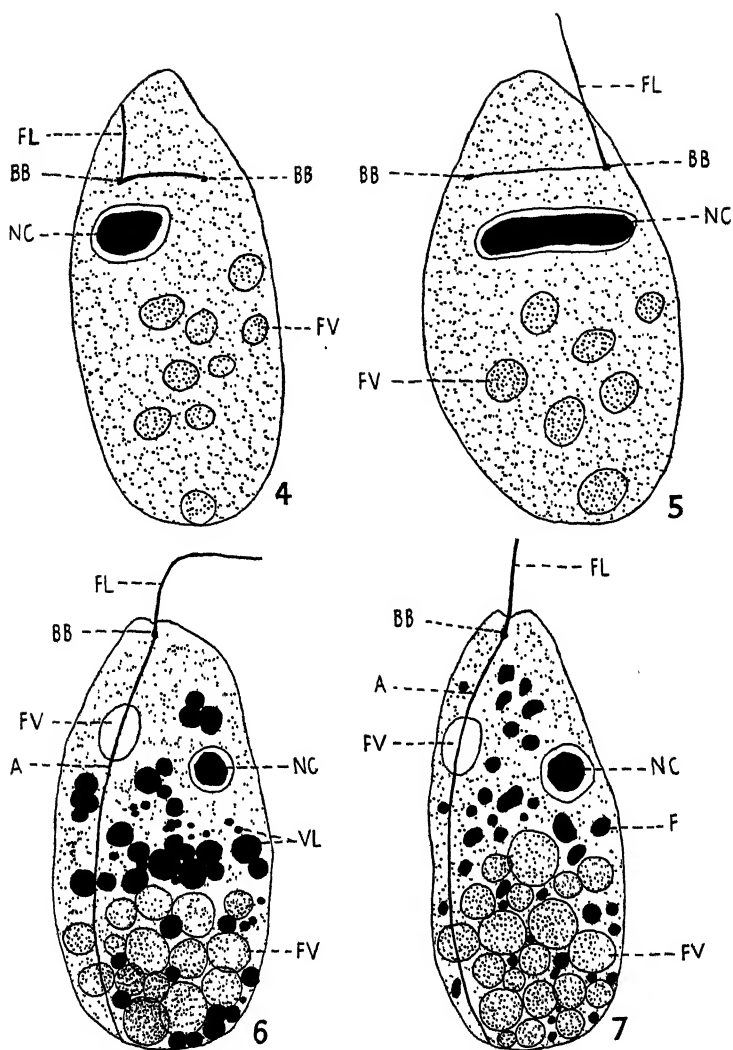
TEXT-FIGS. 4–7.

Copromonas.

Figs. 4 and 5.—Stages in division showing division of basal body (centriole).

Fig. 6.—Volutin granules (methyl blue).

Fig. 7.—Sudanophil fat (Sudan IV).

A., axostyle; B.B., basal body (centriole); F., sudanophil fat; F.V., food vacuole; N.C., nucleolo-centrosome; V.L., volutin.

granule, and the movement of these granules to the anterior end of the individuals. Fig. 13, Pl. 47, is the stage when two axostyles are very clearly seen. We have seen two axostyles in later stages than those indicated in fig. 13, Pl. 47.

Our preparations do not lead us to agree with Wenyon (1926) that basal granules go as far down, during division, as figured by him. In undividing monads we have always seen the basal granule at the anterior end of the organism, as we have shown in our diagrams.

Text-fig. 7 shows the distribution of the sudanophil fatty substances in Copromonas subtilis. They are generally irregular lumps distributed throughout the cytoplasm. Volutin granules often lie in the posterior region of the organism (Text-fig. 6) and are stained in neutral red, as has been claimed by Miss Patten and Beams (1936) and others in different flagellates. Mitochondria are generally elongated in shape and are beautifully revealed by both silver and osmic methods (Text-fig. 8 M). It is interesting to note that in the preparations that were made to study fat and volutin granules the axostyle is a much more prominent structure than by the routine fixatives.

STRUCTURE OF COPROMONAS.

In Text-fig. 8 we have given our conception of the structure of Copromonas subtilis. The flagellum passes into the organism ending in a basal granule B.B. (blepharoplast, centriole, &c.), from which pass down two other structures, a finer, the rhizoplast (R.P.) which forms a connexion with the nucleolo-centrosome (N.C.) of the nucleus. The coarser filament (A) stretches the whole length of the organism and is known as the axostyle. From the gullet or cytostome (C.Y.), a canal, the cytopharynx (C.Y.P.), passes into the upper region of the animal, and often has an ovoid food vacuole (F.V.) attached to it. When the food vacuole becomes detached from the cytopharynx it assumes a spherical shape. The lower region of the monad is usually crammed with food vacuoles, bacteria, &c., in various stages of digestion. Storage bodies are found throughout the organism in the form of sudanophil fat (F.), shown in black. In addition, so-called volutin granules (V.L.) are found in the
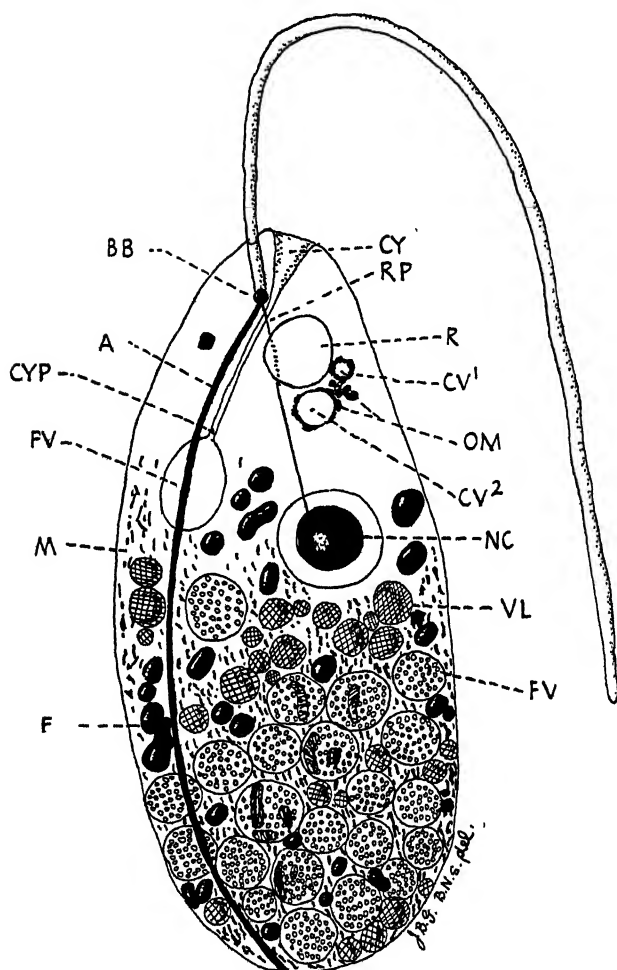
TEXT-FIG. 8.

Plan of Copromonas, slightly diagrammatic. For explanation
see text.

lower region of the cell, and these may be made to stain·vitally
in neutral red.

The osmo-regulatory mechanism of the monad consists of
a reservoir (R.) usually but not always with non-osmiophil walls,

and a number of contractile vacuoles ($c.v^1$, $c.v^2$) which arise from and inside granules of osmiophil material (o.m, Golgi bodies) which lie in this region, and which are carefully divided between the daughter monads during binary fission. There are many mitochondria (m.) lying principally in the lower region of the organism.

SUMMARY OF THE LIFE-CYCLE OF COPROMONAS SUBTILIS, PARTLY AFTER DOBELL (Text-fig. 9).

The adult monad (A) in the upper circle is undergoing asexual multiplication by binary fission, in (B) two reservoirs having arisen probably by swelling up one or two contractile vacuoles. The osmiophil material (g.) forms a mass above the dividing nucleus, which, as the new cell-wall is formed (C), is forced down in front of it and finally divided into two groups (g.), which scatter around the reservoirs as in (D). In stage B, the basal granule has divided into two, the rhizoplast and axostyle have disappeared, and in stage C a new flagellum has begun to grow out of the basal body (b.b.) on the right. New axostyles have grown down from the basal bodies in each individual, and in stage (D) the two daughter monads are ready to separate.

The sexual cycle is shown in the lower circle. Two individuals ready to associate come together by their anterior ends, their osmiophil material (Golgi bodies, g.) fuse, and their nuclei give off polar bodies (reduction bodies) as in (F), which degenerate; one flagellum is withdrawn, and the individuals cease to feed but continue motile. In the next stage another reduction body is given off, and the number of food vacuoles is much reduced. According to Dobell, after the conjugation of the matured nuclei the new individual may either pass back to stage (A) or may pass on to encystment as in stages (I), (J), and (H). In both (I) and (H) the nuclei are fusing, and in stage (J) the cyst-wall has formed. The osmiophil material (g.) and nucleus (n.) are shown.

DISCUSSION.

Elsewhere (1938) it has been pointed out that in the choano-flagellate the posteriorly situated contractile vacuoles are quite
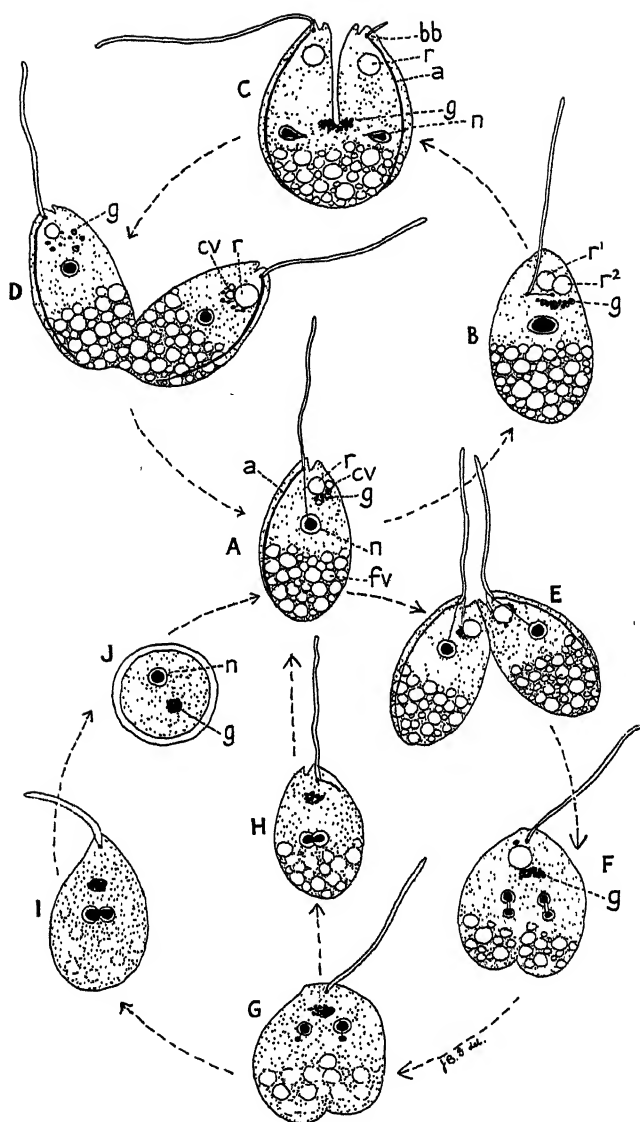
TEXT-FIG. 9.

Life-cycle of Copromonas, showing nucleus and Golgi apparatus,
&c. For explanation see text.

a., axostyle; *cv.*, contractile vacuoles arising within osmiophil (Golgi)
material; *bb.*, basal body of flagellum; *g.*, osmiophil material
(Golgi apparatus); *fv.*, food vacuole; *n.*, nucleus.

separate from the parabasal (Golgi apparatus) which lies below the flagellum. It is certain, too, that in a ciliate like S p i r o-s t o m u m the contractile vacuole wall is not osmiophil, and true Golgi bodies are scattered in the ground cytoplasm. The same appears to apply to B l e p h a r i s m a, which has been studied by Miss I. Moore (1934). It seems certain that among some Protozoa contractile vacuoles are not necessarily associated with osmiophil material. In P a r a m o e c i u m, C h i l o-m o n a s, N a s s u l a, D o g i e l e l l a, &c., there can be no doubt that osmiophil material is an intimate part of the osmo-regulatory pump (Kitching). In C h i l o m o n a s, Nassonov (1924) was the first to show that the contractile vacuole wall blackened densely after treatment in osmium tetroxide solution. In the present paper we have shown that the osmiophil material is divided between the two daughter flagellates and forms a remarkable picture at the telophase of division (fig. 18 G, Pl. 48). Sigot (1931), working on E u g l e n a, states that, 'au moment de la division du Flagellé, l'ensemble de l'appareil (his "plaquettes osmiophiles") se divise, cette division se produit en même temps que celle de la cinétide et avant celle du noyau, sans que nous puissions préciser si chaque élément se clive à ce moment ou s'il se produit simplement un partage des corps osmiophiles existants entre deux cellules filles. Nous avons vainement essayé de colorer ces éléments au rouge neutre et au vert Janus.'

We agree with Sigot that what we call the Golgi apparatus of flagellates does not colour in neutral red, is divided between the daughter cells in the same manner as the Golgi apparatus of higher forms, and is as characteristically osmiophil as the Golgi bodies of Metazoa. There is however no reason for supposing that the neutral red staining volutin granules have any connexion or homology with the metazoon Golgi apparatus.

We have not had the opportunity of examining the division stages of the Golgi apparatus of E u g l e n a, but in C o p r o-m o n a s the ultimate separation of the osmiophil material takes place usually after the daughter nuclei have completely separated. We believe that the basal granule of the flagellum

(centriole) takes no part in dictyokinesis (division of the Golgi apparatus).

There is a tendency, less noticeable in recent years, for those who have not utilized current Golgi apparatus methods to decry all work done by osmic acid and silver. As we have pointed out above, the osmiophil material is developed on the site of the contractile vacuoles or reservoir, and moves down the cell and becomes divided between the daughter organisms. There can be no doubt that the blackening by osmium tetroxide marks clearly the presence of a definite amount of lipoid substance, which undergoes definite changes during the life of the organism and which is identical with the osmiophil material found in all metazoon cells.

Regarding the relationship between the reservoir and contractile vacuoles, we have already expressed our views (p. 574). The senior author finds it difficult to accept Dobell's (1908) account of the division of the reservoir into two parts prior to the division of the organism. It is quite true that we have found many examples where two vacuoles of reservoir size are present just before the onset of division (figs. 5, 8, Pl. 46, and 10, Pl. 47), but these appear to have arisen by the sudden growth of contractile vacuoles and not by division of a pre-existing reservoir. It is difficult to understand how a thin-walled vesicle filled with water could divide into two. We admit that this point needs further elucidation, and one of us is at present engaged on this problem in Euglena. Sigot throws no light on the point.

Regarding the nature of the euglenoid accessory contractile vacuoles (Text-fig. 2) of Borradaile and other well-known teachers, and the osmiophil canals of Paramoecium (Nassonov), we believe that if the accessory vacuoles were drawn out around stiff canals as in the ciliate we should have an exact homology and resemblance between the two. Thus we feel that the osmiophil canals of Paramoecium are drawn out contractile vacuoles which empty into a reservoir (contractile vacuoles of Paramoecium).

Nassonov's Homology of Contractile Vacuole and Golgi Apparatus.

Nassonov (1924) says, 'The primitive form of the excretion apparatus of Protozoa resembles a bladder with osmiophil walls, whereas this structure in more highly organized Protozoa can assume a more complicated form. The simplest form of the Golgi apparatus must be assumed to be a bladder (vacuole) with osmiophil walls, or a scale (as in germ and some somatic cells of the lower animals); this primitive form can become more complicated in the somatic cells of higher Metazoa, and assume the form of a net.'

Recently it has been shown by one of us (B. N. S.) in Amoeba proteus, and by Mrs. Lamont in Nebela collaris, that no Golgi apparatus is present in these forms. Moreover, the contractile vacuoles of these and many other Protozoa have no osmiophil walls. In the choanoflagellates (Saedeleer, 1930), it seems that an osmiophil Golgi apparatus (parabasal) is present at the base of the flagellum, and true contractile vacuoles may be found far removed at the posterior end of the organism. We believe that the true Golgi apparatus first arose in the flagellates in connexion with the base of the flagellum, and in such forms as Copromonas has secondarily become associated with the contractile vacuole (Gatenby, 1930). We find it impossible to accept Nassonov's homology in the form in which he has stated it, not only because contractile vacuoles can exist without osmiophil Golgi material, but because in such forms as Spirostomum a true Golgi apparatus exists quite separately from the contractile vacuole, which itself has non-osmiophil walls.

We should note that the Golgi apparatus of the trophozoites of various Sporozoa bears a striking resemblance to that of Copromonas under certain conditions, a fact which seems to support the view which has in the past been held by some protozoologists, that the Sporozoa are derived from the Flagellata.

In the choanocyte or choanoflagellate, the Golgi apparatus is a bead lying near the basal granule of the flagellum, and the

reason for its constant position here, and its exact function, are quite unknown. It might be suggested that it either produced by segregation from the ground protoplasm substances necessary for the continual operation of the flagellum, or that it in some way assisted in the elimination of the waste products produced by the violent movements of the flagellum. The latter suggestion would seem more likely, as the subsequent association between osmiophil substance and contractile vacuole would naturally assist in the process. This association we believe has already taken place in Copromonas, but not in the choanoflagellata. In the case of the sponge, we do not yet know whether such fresh-water forms as Spongilla have contractile vacuoles in their choanocytes. From this hypothesis of the osmiophil material becoming associated with the osmo-regulatory mechanism of the cell, one of us (Gatenby, 1938) has suggested that the function of the Golgi apparatus of metazoon cells is that of dehydrating secretory products.

Summary.   .

1. In Copromonas subtilis, Dobell, and Euglena sp. there is a Golgi apparatus consisting of osmiophil material in the form of granules, which are associated with the osmo-regulatory mechanism of the cell.

2. Inside the granules, water collects, so that they become spherical vacuoles, identical with what have in the past been called contractile vacuoles (Copromonas) or accessory contractile vacuoles (Euglena viridis).

3. In Euglena viridis, the Golgi apparatus is closely applied to the so-called contractile vacuole, and consists of numerous loaf-shaped osmiophil bodies which undergo a regular series of changes from systole to diastole, and vice versa.

4. In Copromonas, the osmiophil material may form a thick cortex surrounding what has been called the reservoir, it may be attached to the reservoir in fairly regular loaf-shaped bodies as in Euglena, or it may be completely detached from the reservoir.

5. The so-called contractile vacuoles of Copromonas are

vesicles containing water, which are formed on the site of the osmiophil granules.

6. As far as we are able to say at present, the reservoir of Copromonas is indistinguishable from an enlarged contractile vacuole, and new reservoirs probably arise from swollen contractile vacuoles. It is difficult to believe that the reservoir divides into two, as has been claimed by Dobell.

7. During division of Copromonas, two reservoirs can nearly always be found in the early stages before the nucleus becomes dumb-bell shaped. These seem to have originated from the osmiophil vacuoles.

8. The remaining osmiophil material, when present, moves slightly down the cell, occupying a place in the mid-line. When the new cell-wall between the two organisms has passed down, about one-third the length of the dividing monad, the osmiophil material splits into two sub-equal groups and is so divided between the two organisms. There is therefore a definite dictyokinesis to be found in Copromonas.

9. Just at or after this period, the osmiophil material may become scattered about the upper middle and upper region of the dividing monads, but finally becomes situated in the region of the reservoir.

10. The osmiophil material (Golgi apparatus) persists throughout conjugation and encystment, even when a reservoir cannot be found.

11. There is a rhizoplast joining the basal granule of the flagellum with the intra-nuclear nucleolo-centrosome, and an axostyle is present, passing from the basal granule to the posterior end of the organism.

12. During cell division, the basal granule divides into two and appears to lose its connexion with the two nucleolo-centrosomes of the dividing nucleus. The axostyle appears to be absorbed in the early stages of division and cannot be stained at this epoch, but reappears in each moiety of the dividing organism, when the nucleus is dumb-bell shaped. It appears to reform when the two basal granules have taken their definitive position at the anterior end of the cells.

13. We agree with Wenyon that one flagellum passes over

intact to one of the daughter cells at division, the other flagellum arises from the other basal granule.

14. Numerous fat granules are found throughout the organism; what have been called volutin granules in other Protozoa are present in C o p r o m o n a s, and stain in neutral red.

15. Mitochondria are present mainly in the posterior region of the organism.

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DESCRIPTION OF PLATES 46–48.

LETTERING.

A., axostyle; B., basal granule (blepharoplast); C.V., contractile vacuole; E., food vacuole forming; G., Golgi apparatus (osmiophil substance); G.X., special part of Golgi apparatus referred to in text; N., nucleus; R., reservoir; R.G., reservoir with thick osmiophil cortex.

Figs. 1–3.—Upper region of Euglena sp. prepared by Weigl method and slightly bleached (preparation by Miss R. Patten (1936)). Fig. 3 has been ultra-centrifuged, the reservoir being absent.

Figs. 4–15.—Copromonas subtilis Dobell, prepared by an osmic method (Weigl or Kolatchew). Figs. 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 are division stages.

Figs. 16–19.—Photomicrographs of Copromonas at rest (fig. 16) and in stages of division (figs. 17–19). In fig. 19 the left-hand Golgi apparatus has floated down below that in the right-hand daughter organism (Kolatchew method).

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Gatenby and Singh
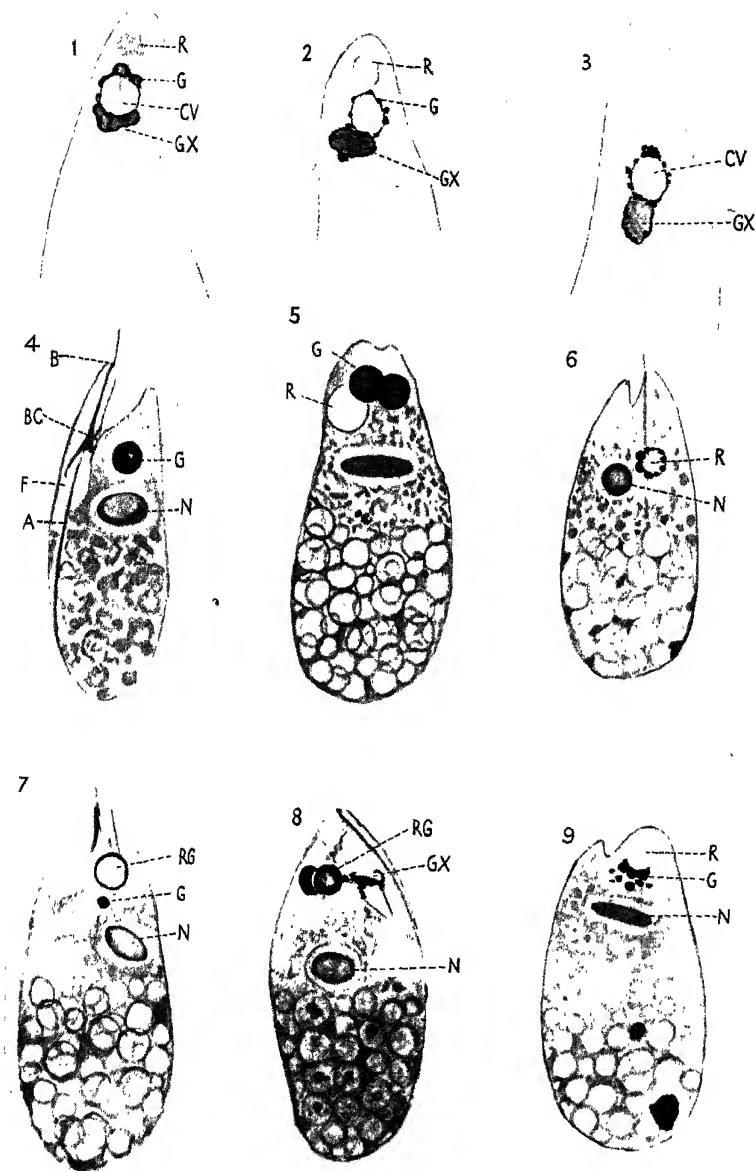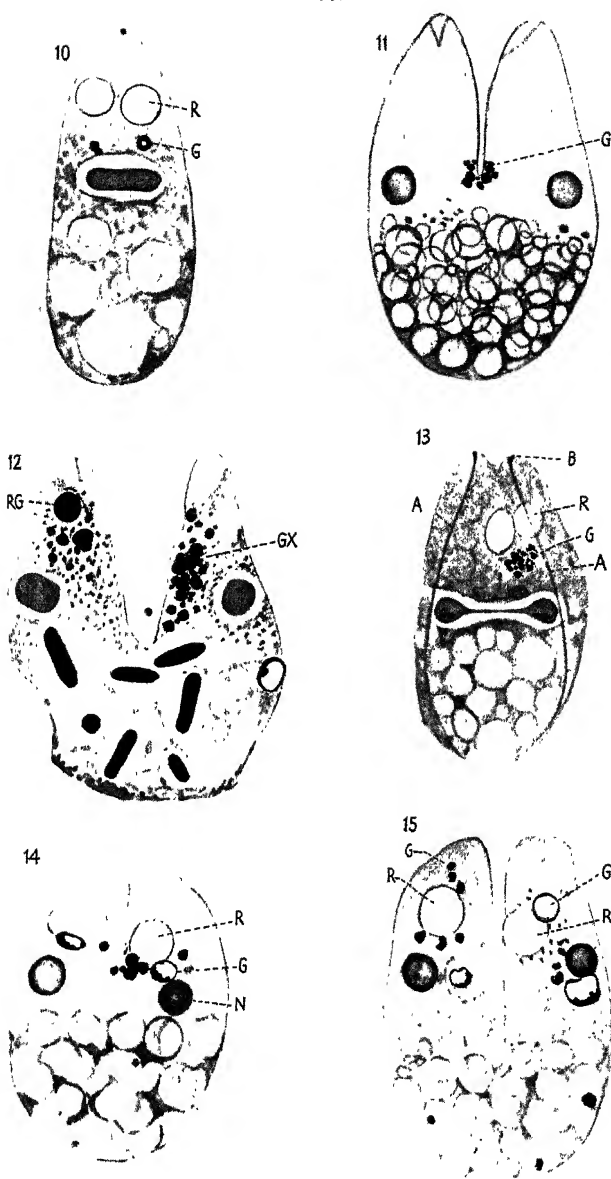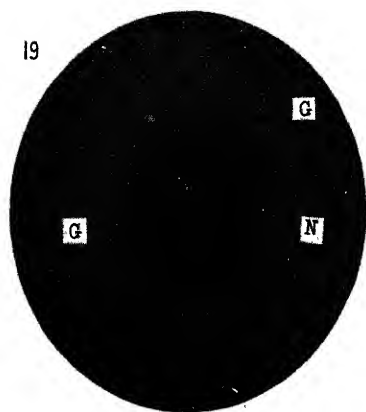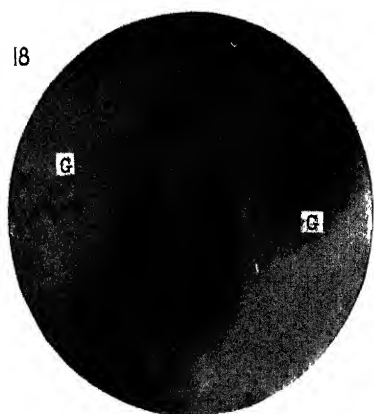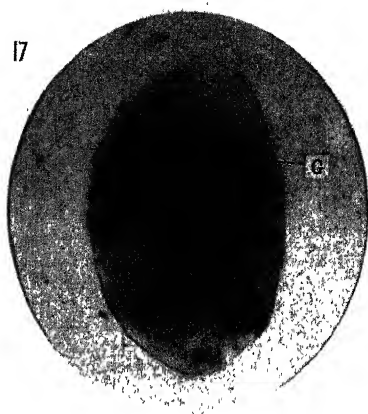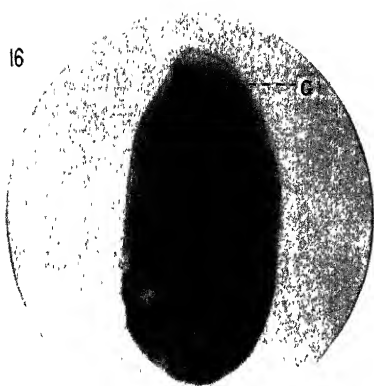
The Spermatheca of Loligo vulgaris.

I. Structure of the Spermatheca and function of its Unicellular Glands.

By

G. J. van Oordt.

Department of Experimental Morphology, Zoological Institute,
University of Utrecht.

With Plate 49 and 1 Text-figure.

1. INTRODUCTION.

IN the Decapod Cephalopods the transfer of the spermatophores from male to female takes place with the aid of the hectocotylus, the left ventral arm of the male.

There are two methods of copulation in the American squid Loligo pealii. According to Drew (1910), the spermatophores are inserted into the mantle chambers of only those females which are about to deposit their eggs. The male swims by the side of such a female, with his head in the same direction. Just before attachment, he sinks slightly beneath her and grasps her body with his arms; the hectocotylus then seizes the spermatophores which appear in the opening of the funnel, and with a rapid sweep these are immediately inserted into the mantle chamber of the female. After about 5 or 6 seconds, this arm is withdrawn; the animals separate almost immediately and in another 5 or 6 seconds the empty cases of the spermatophores pass out of the funnel. The sperm-reservoirs are found later to be attached near the end of the oviduct (Drew, 1910, pp. 329–33). The secretion of glands of the hectocotylus possibly helps to attach the spermatophores to each other and to the hectocotylus during their transport into the mantle chamber of the female.

Although the female of Loligo pealii may not yet be ready to deposit her eggs, she is capable of copulation. According to Drew (1910, pp. 333–4) the sperm-reservoirs are under

such circumstances ejaculated from the spermatophores, and then attached to a receiving depression of the buccal membrane of the female. This type of copulation does not take place between a male and female, united parallel, but when facing each other. When the animals separate, the empty cases of the ejaculated spermatophores are held for several minutes between the arms of the female and are then finally dropped. The sperm-reservoirs are attached by cement, carried inside the spermatophores,[1] and liberated by the ejaculation.

The depression which receives the sperm-reservoirs is situated at the anterior side of the spermatheca; the latter possesses the shape of a mamma, having a distinct nipple, the anterior tip of which bears the opening of the spermatheca. This whole structure is found at the inner side of the buccal membrane, and is therefore in the swimming animal, situated ventrally to the mouth.

According to Verwey (unpublished observations) the spermatophores of specimens of Loligo vulgaris, caught off the coast of Holland, are never deposited in the mantle chamber of the female, but are always attached to the depression near the wall of the spermatheca or to the wall itself.

As Drew has already described, the spermatozoa become stored in the spermatheca. The structure and size of this organ were described by him in outline, but its physiology is still practically unknown. Little or nothing is known of the following points: (1) the factors which play a part in the transit of the seminal fluid from the sperm-reservoirs into the spermatheca; (2) the function of the unicellular glands, found almost everywhere at the inner side of the spermatheca; and (3) the function of the large ganglion lying near the spermatheca.

2. Material and Technique.

The squid furnishing the material for the present investigation is Loligo vulgaris L., which is obtainable every spring in large quantities from the North Sea in the Zoological Station at Den Helder. It is very easy to get here abundant fresh

[1] For further particulars concerning the spermatophore and its ejaculation cf. Drew (1919).

animals, but living, healthy, undamaged specimens are only available in small numbers.

All spermathecae were fixed in Bouin's fluid, and stained with haemalum and eosin in the ordinary way, or with van Gieson's stain for the distinction of connective and muscle tissue.

3. THE STRUCTURE OF THE SPERMATHECA.

As already mentioned, the spermatheca has mostly a mamma-like shape, with a distinct nipple, on which the entrance to the interior of the spermatheca is found (fig. 1, Pl. 49). The wall of the spermatheca consists, contrary to the body-wall, of a deeply staining high columnar epithelium, covered by a mass of rather hard material, the cuticle; the latter is thicker in the region of the depression (where the sperm-reservoirs are mostly attached) and is evidently secreted by the epithelial cells. In many cases, sperm-reservoirs are found outside this depression; according to Drew (1910, p. 335) the spermatozoa, escaping from such reservoirs, do not find their way into the spermatheca.

The wall of the spermatheca (fig. 2, Pl. 49) consists further of a rather thick layer of connective tissue and muscle-fibres. The lumen of the spermatheca is divided by several partitions, outgrowths of the connective tissue and muscle sheath, into a number of primary alveoli, which in their turn are subdivided into secondary alveoli; the latter are lined by a cubical epi-thelium at their base and by a cubical epithelium intermixed with numerous secretory goblet-cells in the region near the entrance to the spermatheca. In the interior part of the alveoli, especially near the wall of the spermatheca, large quantities of spermatozoa are stored. When a spermatheca is opened, and its contents examined microscopically, it is found that the spermatozoa are inactive in the alveoli.

4. FUNCTION OF THE SECRETION OF THE GOBLET-CELLS.

In order to determine how far the spermatozoa of Loligo enter the spermatheca owing to the attraction of a chemical substance secreted by the goblet-cells, or how far the seminal fluid is sucked into the spermatheca by the action of the

muscles of the spermatheca-wall, an examination was made of the motility of the spermatozoa in a pulp of the wall of the spermatheca and its contents.

In the sperm-reservoirs the spermatozoa are motionless, but when discharged from the reservoirs into sea-water they im-
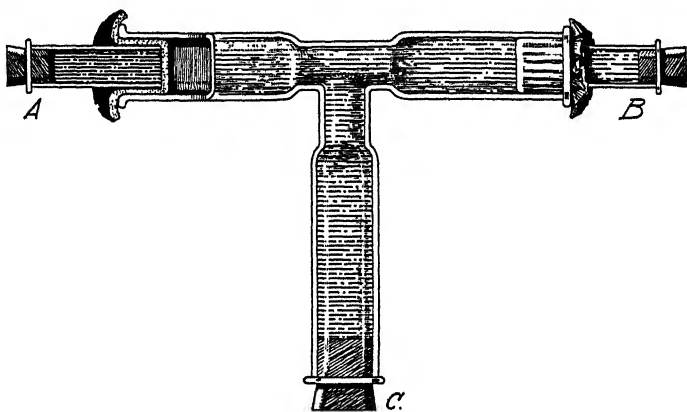
TEXT-FIG. 1.

Apparatus used for the observation of the motility of Loligo-spermatozoa. A, tube filled with contents of the spermatheca. B, tube filled with sea-water; C, cork.

mediately become very active. Spermatozoa were transferred into a T-shaped tube (Text-fig. 1), filled with sea-water; one of the openings of which was closed by a cork (c), the other two openings, however, were closed by small tubes covered with pieces of chamois, impregnated with sea-water. One of these small tubes (A) was filled with a pulp of the wall of a spermatheca, the other (B) contained only sea-water. Under the microscope it could be clearly seen that the spermatozoa (very active in pure sea-water) became quiet in a few minutes when in the neighbourhood of the small tube (A), containing the secretion of the spermatheca-wall. It seems clear that this secretion does not attract spermatozoa; on the contrary, the latter are immobilized by the action of a substance which must have passed through the chamois-cover. This also becomes evident when a

small quantity of the contents of a spermatheca is transferred to sea-water containing very active L o l i g o -sperm; the spermatozoa are then totally immobilized after 5 to 10 minutes. From these simple experiments it followed that chemotaxis is not the cause of the spermatozoa entering the spermatheca.

When the spermatozoa have entered this structure they become inactive after a few minutes. The experiments of Gray (1928) have shown that the activity of sea-urchin spermatozoa is due to the grade of dilution of the seminal fluid. From my experiments it does not seem likely that, in the squid, mechanical overcrowding can be the origin of the inactivity of the spermatozoa in the spermatheca.

Lack of living healthy squids made it impossible to determine in v i v o how the seminal fluid, discharged from the sperm-reservoirs, is taken up by the spermatheca. The structure of its wall, in which many muscle-fibres are found, makes it probable that the seminal fluid is sucked up by the spermatheca by means of movements of its muscles. This is also suggested by an observation of Drew (1910, p. 346) that the spermatozoa enter the opening of the spermatheca in a narrow stream, and with the tails all turned in the same direction, i.e. the heads going first and the tails trailing behind; my own observations show that, sometimes, a whole sperm-reservoir may be found in the interior of the spermatheca.

The above experiments clearly show that the secretion of the inner epithelium of the spermatheca has an immobilizing effect on stored spermatozoa. The function of this organ therefore may be compared to that of the mammalian epididymis. In this gland the spermatozoa are also preserved, till they are ejaculated during coitus (von Lanz, 1924, 1926, 1936). Moreover, it was found (von Lanz, 1929) that the acid reaction of the secretion of the epididymal epithelium renders the spermatozoa quite inactive.

The hydrogen-ion concentration of the contents of the spermatheca had to be determined. The small size of the spermatheca made it impossible to determine the hydrogen-ion concentration of its contents by means of the more usual laboratory methods. In the Laboratory of Comparative Physiology at the University

of Utrecht, the pH of the interior of the spermatheca (sent from Den Helder to Utrecht) was measured by the glass-electrode-method, and found to be 6·06. A pulp of the spermatheca-wall in distilled water showed a pH of 6·20. The contents of the Loligo-spermatheca are thus definitely acid, as is the case with the contents of the mammalian epididymis (von Lanz, 1929). When, moreover, the acidity of sea-water in which large quantities of Loligo-spermatozoa are active, is artificially brought to the same level as that of the spermatheca contents, the sperm shows less activity after 5–10 minutes. These observations, therefore, make it highly probable that Loligo-spermatozoa, stored in the spermatheca, are immobilized by the acid reaction of the secretion from the inner epithelium of this organ.

5. SUMMARY.

The structure of the spermatheca of Loligo vulgaris is described; it lies on the inner wall of the buccal membrane and within it large quantities of inactive spermatozoa are stored.

This inactivity of the spermatozoa within the spermatheca is attributed to the effect of the secretion of the goblet-cells, situated as unicellular glands on the inner wall of the spermatheca.

Inactive spermatozoa from the spermatheca become very active in sea-water, but are immobilized again after a few moments' contact with the pulp of the spermatheca contents.

The hydrogen-ion concentration of the spermatheca contents is approximately 6·06; and, since spermatozoa become inactive in sea-water, the hydrogen-ion concentration of which is increased to this level, it seems probable that the inactivity of the spermatozoa within the spermatheca is due to the presence of hydrogen-ions.

The spermatheca is functionally comparable to the mammalian epididymis.

6. ACKNOWLEDGEMENTS.

and to Dr. J. Verwey, director of the Zoological Station at Den Helder for providing the material during my stays at this station and for giving much information on the habits and life-history of the common squid.

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EXPLANATION OF PLATE 49.

Fig. 1.—A median section of the spermatheca of Loligo vulgaris. *Conn.musc.*, sheath of connective tissue and muscle-fibres; *depr.*, depression for attachment of the sperm-reservoirs; *sperm.*, spermatozoa, stored in alveoli of the spermatheca. (× 18).

Fig. 2.—Part of the wall of the spermatheca. (× 120.)
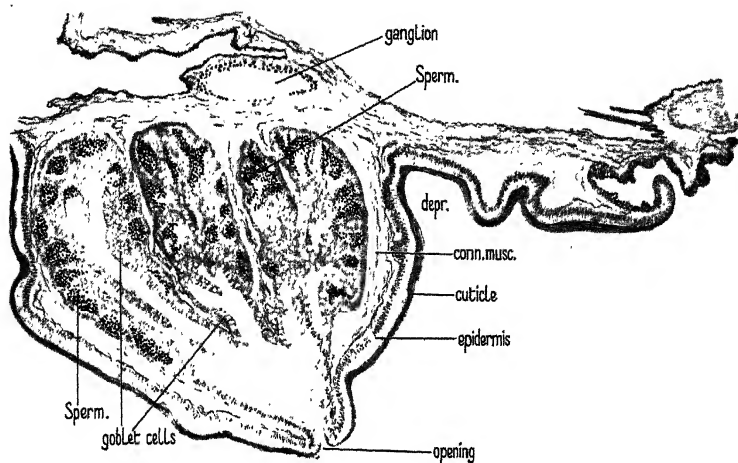
FIG. 1
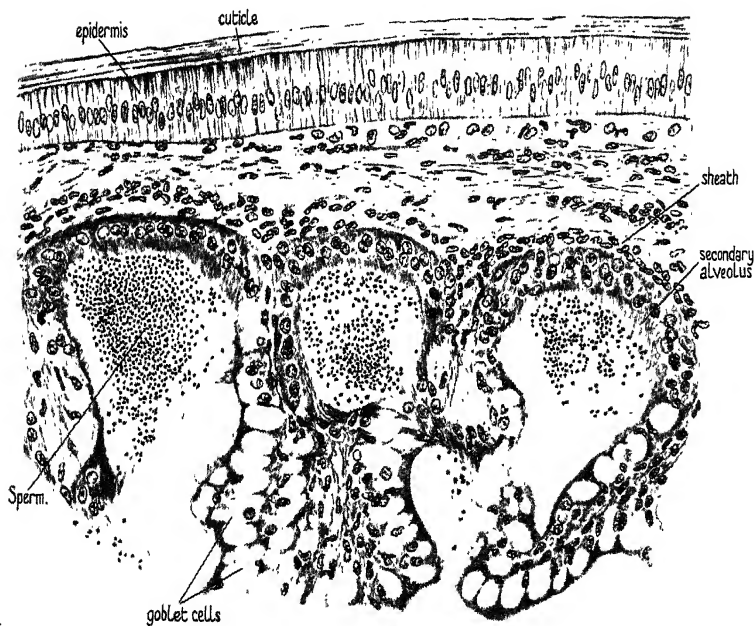
FIG. 2

The Cytology of Amoeba proteus 'Y' and the effects of large and small Centrifugal Forces.

By

B. N. Singh, M.Sc. (Allahabad)

(Department of Zoology, Trinity College, Dublin.)

With Plates 50–51 and 2 Text-figures.

Contents.

1. Introduction and Previous Work.

The development of the air-driven ultra-centrifuge by J. W. Beams and his associates (1930–3) has proved a valuable means of discriminating between the pre-existing cytoplasmic bodies and those that are fixation artefacts. Ultra-centrifuging has very clearly shown that the Golgi apparatus is not an artefact produced by the action of fixatives on cytoplasmic colloids. It is also a very satisfactory means by which to distinguish between the pre-existing neutral-red bodies and those produced by the segregation of the dye. One can very easily distinguish, by the above method, the separate identity of the Golgi bodies from neutral-red staining bodies where both types of inclusions occur side by side. In the Sporozoa, where the true homologue of the Golgi apparatus of the Metazoa has been clearly shown to exist by different workers, one can very easily identify

the separate Golgi bodies and neutral-red staining bodies by centrifuging, and we may also ascertain whether the latter bodies are pre-existing, or artefacts produced by the neutral-red staining.

As Amoeba proteus is very often used as a type of simple cell, it was thought that the detailed study of its cytology and the effect of centrifuging would be of interest, and might shed some further light on the question of the homology of the protozoan and the metazoan Golgi apparatus. It was also thought possible to ascertain whether any homologue of the Golgi apparatus exists in Amoeba proteus as claimed by Causey (1925), Hirschler (1927 a), Hall (1930 b), Hall and Loefer (1930), Nigrelli and Hall (1930), and V. E. Brown (1930) in the Protozoa Sarcodina. A little work has been done on this subject by V. E. Brown (1930), who claims to have demonstrated two types of Golgi bodies in Amoeba proteus. One of these is composed of granules and the other of spherules with black rims (after the Mann-Kopsch fixative). After Champy fixation and staining with acid fuchsin, thionin, and aurantia, he found that the spherules are light blue with dark blue rims. According to him, the spherules when viewed at a central focus appear as rings and crescents having clear centres. The contractile vacuole does not blacken with osmic acid, and in no case are the Golgi bodies associated with it. He found small granules grouped around the contractile vacuole which do not take osmic stain, but are revealed by acid fuchsin. In addition to these he finds small vacuoles of variable size which are attached to the supposed Golgi bodies, and are also found in the endoplasm, and they are numerous around the contractile vacuole. He believes that the small vacuoles originate from small granules which move about in the cytoplasm and are grouped around the contractile vacuole. They flow together to form the new vacuole after systole.

Brown thinks that it may be possible that the black granules, which are of variable size, and which he identifies as Golgi bodies, grow in size to form black spheres with black rims, and compares these two types of Golgi bodies with those observed by Hirschler (1914), King and Gatenby (1923), Joyet Lavergne

(1926), and Hall (1930 b). He did not use silver methods to confirm his results, and has not drawn mitochondria in his diagrams, to avoid confusion with the various types of bodies that he has described. According to Brown no network exists in Amoeba proteus, so he is led to believe that in Protozoa secretion is a constant process and, therefore, it is natural to expect globules, which are hypertrophied Golgi network. He believes that the relationship existing between crescent-shaped Golgi bodies and the small vacuoles which are present throughout the endoplasm, and are also attached to the supposed Golgi crescents, is the same as pointed out by Bowen (1926) in the case of gland-cells.

Sister Carmela Hayes (1924) pointed out that the nutritive spheres in Amoeba proteus, as so termed by Sister Monica Taylor (1924), are of the nature of glycogen and not of fat, for which she has given various reasons. She found abundance of minute starch grains by staining amoebae with a solution of iodine in potassium iodide. When amoebae containing nutritive spheres were crushed under a coverslip and treated with iodine solution, the small nutritive spheres stained dark brown and the large ones stained less deeply. The big spheres seem to form centres around which starch granules collect. The nutritive spheres become yellow to reddish brown after treatment with iodine, and red after Best's carmine test for glycogen. The Delafield's haematoxylin and light green method stains nutritive spheres purple. Sister Carmela Hayes noted that in smear preparations the spheres lost their spherical form and became irregular patches which stained deeply in thionin and Delafield's haematoxylin.

Recently a detailed account of the cytology and the effects of centrifuging has been given by Mast and Doyle (1935 a and 1935 b). They used Amoeba proteus 'Y' and Amoeba proteus 'X' (Amoeba dubia) in their work. According to them the cytoplasmic bodies are: alpha granules, beta granules (mitochondria), crystals (lying inside crystal vacuoles), fat, and a single contractile vacuole.

By centrifuging, the cytoplasmic bodies are stratified in different layers. Beginning with the aggregation of fat-globules

at the extreme centripetal end in the cell, the other cytoplasmic bodies, except the alpha granules, are stratified as follows: (1) crystal vacuoles without crystals; (2) crystal vacuoles with very small crystals and the contractile vacuole; (3) hyaline and very finely granular substance; (4) the nucleus and the food vacuoles containing much fat and little starch; (5) crystal vacuoles with crystals of moderate size; (6) crystal vacuoles with large crystals, mitochondria, and food vacuoles containing little if any fat; (7) refractive bodies.

The alpha granules were more abundant in the centrifugal half than in the centripetal half of the organism. They have shown that the crystals lie in the centrifugal side of the crystal vacuoles, and the chromatin, being heavier than the karyolymph, collects at the centrifugal side of the nucleus. There is a thick layer of substance, lying at the centrifugal side of the contractile vacuole, with beta granules adhering to it, and only a thin layer at the opposite side, with no beta granules. This shows that both beta granules and the substance surrounding the contractile vacuole collect at the centrifugal side of the vacuole.

By the use of the microscope centrifuge they observed that some of the amoebae had no pseudopodia and were elongated, while others had various numbers or sizes of pseudopodia. The fat-globules formed permanent aggregations in the pseudopodia and sometimes the tips of these pseudopodia were separated from the rest of the body. In support of this, they point out that many amoebae, which were fixed after centrifuging, had no fat. After the stratification of the cytoplasmic components, the amoebae continued to elongate, and in doing so, the central portion became smaller and smaller till the two ends rapidly separated.

The alpha granules are approximately 0·25 of a micron in diameter, and are so near the limit of microscopic vision that they were neither able to make out the structure of these granules in the living condition, nor were they able to stain or identify them in fixed preparations.

The beta granules (mitochondria) are about 1 micron in diameter, distributed uniformly throughout the cytoplasm, and

form a layer on the surface of the contractile vacuole. These granules are nearly spherical or ellipsoidal in the living condition. They change in form, and protuberances like pseudopodia appear at intervals. Sometimes the spherical ones become ellipsoidal or rod-like in form and vice versa. With Janus green solution, both rod-shaped bacteria and mitochondria are stained a green colour. Due to the action of the fixatives, mitochondria become more elongated.

Mast and Doyle found that the organisms, from which all the mitochondrial granules are removed, die after 12 hours, showing that these bodies are essential for life. The increase in number of the mitochondrial granules was seen in those organisms from which only 15 per cent. of them were removed. They were not able to make out whether the beta granules arise d e n o v o or by the division of the pre-existing ones. By changing the number of beta granules by cutting off different portions of the amoebae, they noted the rate of elimination of the fluid by the contractile vacuole, and thus came to the conclusion that the frequency of the contraction of the contractile vacuole varied directly with the number of beta granules per unit volume of cytoplasm. Thus, according to the above conclusion, they point out that the beta granules function in the accumulation of the fluid eliminated by the contractile vacuole, i.e. the beta granules function in excretion. They support Metcalf's (1910) idea that the surface of the contractile vacuole is covered with a layer of granules which function in excretion. They say, 'This conclusion is supported by the facts that the contractile vacuole and beta granules in amoebae stain like Golgi substance and mitochondria in secretory cells in Metazoa, and are associated in the same way as these two structures in these cells (Nassonov, 1924, 1926; Brown, 1930; Mast and Doyle, 1935 a).'

They centrifuged amoebae and cut them in such a way as to reduce the number of refractive bodies and crystals, and kept such amoebae in culture solution containing Chilomonads. By studying for various lengths of time a number of such specimens, paying special attention to the location and movement of the beta granules, they came to the conclusion that the beta

granules function in transporting enzymes to the food vacuoles, and in transferring digestive substances from the food vacuoles and crystal vacuoles to the refractive bodies, and that the substance of the refractive bodies present in the food vacuoles is used up in the development of the refractive bodies present in the cytoplasm.

Both plate-like and bipyramidal crystals are present in Amoeba proteus, and are suspended in the fluid in the crystal vacuoles. This fluid is alkaline, because it becomes reddish yellow in neutral-red solution. Usually one crystal is suspended in each vacuole, and the size of the vacuole in relation to the crystals varies greatly. In some cases Brownian movement of the crystals is well marked. The plate-like crystals are optically active and are probably lucine. The bipyramidal crystals are not optically active and contain a magnesium salt of a substituted glycine. They traced the formation of the crystals from the food in the food vacuoles. The vacuoles in which the crystals lie are formed by the division of the old food vacuoles. According to them the crystals are formed from the amino acid derived from food during the process of digestion. The substance in the crystals is used up in the formation of refractive bodies, because the increase in number of the refractive bodies is followed by the decrease in the number of crystals.

About 5 per cent. of the crystals possess blebs, which are globular in form. The size of these blebs varies greatly, and the largest are nearly as large as the crystals, and the smallest are barely visible. The blebs stain intensely blue with Nile blue, crimson in neutral red, and become black in osmic acid. The small blebs are definitely blackened in 30 minutes, while the large ones require longer time. The former dissolve in trichloracetic acid, while the latter do not. Thus they came to the conclusion that the composition of the blebs changes during development. The small ones have properties similar to those of vacuole refractive bodies; the large ones to those of the outer layer of the refractive bodies. They point out that the blebs are formed from the vacuole refractive bodies, and give rise to the outer layer of the refractive bodies. By keeping amoebae in 5 per cent. calcium gluconate mixed in culture

solution, the blebs on the crystals are enormously increased in number, and thus they came to the conclusion that the formation of blebs is due to the absorption of the substance from the culture solution, and this substance comes from vacuole refractive bodies.

The refractive bodies in the cytoplasm differ from those present in the food vacuoles, so they call the former refractive bodies, and the latter vacuole refractive bodies. When amoebae are kept in 1/50,000 solution of neutral red the refractive bodies are stained deep crimson. These workers have not mentioned any time taken by the refractive bodies to stain in neutral-red solution. The central portion of the refractive body is lighter than the outer portion, and by applying pressure they came to know that the outer portion consisted of a thick dark crimson layer, and this is the only portion that stains with neutral red. The central portion consists of a spherical fragile shell which surrounds a fluid substance. By increasing the pressure this portion is cracked into several pieces, and a clear fluid comes out of it. The crimson layer of the refractive bodies fuses to form a continuous matrix through which the central colourless mass is scattered. This shows that the outer layer is definitely fluid. By the above method they claim to have proved that the encysted amoebae described by Sister Monica Taylor (1924) are refractive bodies. They point out that the refractive bodies are probably broken by sectioning, and what she called ' chromosomes' were fragments of the shell, what she called 'hatching ferment' was the fluid contained in the shell, and what she called 'cyst' was the outer oily layer.

The outer layer of the refractive bodies did not stain with Sudan dyes, but became intensely blue in Nile blue. With osmic acid it became black and did not readily bleach in hydrogen peroxide or turpentine. They kept amoebae in alcohol and 35 per cent. acetic acid respectively, and found that neither the refractive bodies dissolved nor decreased in size, but after these treatments these bodies did not take Nile blue or osmic stain. This shows that the outer layer is soluble in fat solvent, i.e. a lipide dispersed in a substance which is not lipide. By fixing amoebae in formalin, and then keeping them in methylene blue

in sulphuric acid, or by directly keeping the amoebae in the latter, they found that the outer layer became blue black, showing that this layer contains metachromatin (a substance composed of nucleic acid and an unknown base). Millon's reagent gave the protein reaction. In acetic acid, up to 30 per cent. concentration, the outer layer of the refractive bodies dissolved, but in higher concentrations it did not. Thus they came to the conclusion that the outer layer consists of a protein stroma which is impregnated with a lipide substance.

The shell is a carbohydrate, but they were not able to make out the chemical composition of the fluid inside the shell.

By using the methods for the demonstration of the Golgi apparatus, Mast and Doyle came to the conclusion that the outer layer of the refractive bodies gives positive Golgi tests, and also concluded that the refractive bodies were described by Brown (1930) as Golgi bodies. It could be pointed out that these workers have ignored the rest of the structures described by Brown, particularly the granular type of Golgi bodies, &c.

The so-called Golgi bodies contain two substances, one which becomes black in osmic acid and does not readily bleach in turpentine, hydrogen peroxide, and chlorine water, but dissolves in fat solvents, and the other which does not dissolve in fat solvent, i.e. trichloracetic acid. The former they call 'O' and the latter 'T'. They quote that Beams (1931) and Tanaka (1932) demonstrated that the 'O' substance becomes red with neutral red and Nile blue solutions, and 'T' substance does not stain in these. According to Mast and Doyle, the refractive bodies contain a carbohydrate, and the outer layer consists of a stroma which is impregnated with the fatty substance, therefore these bodies are related to plastids in plant-cells. Thus they maintain that the plastid in plant-cells is the homologue of the Golgi apparatus of animal cells.

It would not be out of place to point out that such a view is not valid, and is not accepted by the majority of workers either in plant or in animal cytology. I think these workers have mainly relied on the work of Weier, who, recently, in a number of papers tried to establish the fact that the plastids in plant-cells are the homologue of the Golgi apparatus. From the discovery

of the osmiophilic platelets by Bowen, which was subsequently confirmed by Gatenby and his pupils (1928), we think that these platelets are the only structure in plant-cells which can be compared with Golgi apparatus. Such a view is correct both from the morphological as well as from the functional point of view as was shown by Bowen in his valuable work. I am personally convinced by seeing a series of preparations made by Miss Jones[1] (1938), that the plastids can never be compared with the Golgi apparatus of animal cells from the morphological point of view, nor from that of the staining reactions. The plastids stain with iron alum haematoxylin and acid fuchsin, while the osmiophilic platelets give the reactions generally found in the case of the Golgi apparatus. Ultra-centrifuging has further been useful in establishing the fact that the osmiophilic platelets in plant-cells are altogether separate from the rest of the cytoplasmic components, as was shown by Beams and King (1935) and which has recently been confirmed by Miss Jones, in this Department. In the present state of our knowledge it is rather difficult to identify exactly the function of the osmiophilic platelets, particularly in somatic cells, but it can be stated that their function may be different in different plant tissues, as we find in the case of the animal-cell Golgi apparatus. Weier (1933) says, 'There is some evidence for considering the platelets to be the primordia of plastids.' This view cannot be accepted because, from evidence provided by the ultra-centrifuge, we know that there is no relationship between the plastids and the platelets.

By removing refractive bodies, Mast and Doyle observed that these bodies did not interfere with the vital process in amoebae, and they originate and develop in cytoplasm in presence of food and function as reserve food.

The vacuole refractive bodies stain more intensely with neutral red than the refractive bodies, and all the substance in them is stained. They do not contain anything in the nature of a shell. In Nile blue they become intensely blue, and black with osmic acid. They are just like the outer layer of the refractive bodies, with the only difference that they do not contain protein

[1] 'La Cellule', t. XLVII, f. 1, 1938.

and are entirely soluble in fat solvents. Mast and Doyle claim that the vacuole refractive bodies stain with neutral red and become black with osmic acid, and therefore these bodies contain a substance which Parat and Hall and others call 'vacuome'. The vacuole refractive bodies originate from food and function as reserve food material. The substance of these bodies as well as of the bipyramidal crystals are used up in the development of the outer layer of the refractive bodies.

The fat-globules in the cytoplasm stain orange with Sudan III in alcohol, red with Sudan III in alcohol acetone mixture, red with Sudan IV in alcohol, maroon with Sudan IV in alcohol acetone mixture, pink with Nile blue sulphate, red with pure Nile blue sulphate osazone, brown to black with osmic acid (brown in 15 minutes and black in several hours), and yellow with chrysoidine. The fat-globules in the food vacuoles also stain with Sudan dyes, osmic acid, and chrysoidine, but they stain blue instead of pink with Nile blue. By keeping amoebae for several hours at a temperature of 5° C., the fat-globules in the cytoplasm crystallized, but those present in the food vacuoles did not. Thus they came to the conclusion that the fat-globules in the cytoplasm are mainly composed of neutral fat, and those present in the food vacuoles contain fatty acids. In staining solutions containing less than 30 per cent. water, the fat-globules are dissolved, and in fixatives containing chromates they are poorly preserved.

By removing fat from the amoebae, and feeding them on Chilomonads, they observed that the fat-globules of the Chilomonads, lying inside the food vacuoles, contain fatty acid which is neutralized and passed into the food vacuoles. Later on these disappear, and then fat appears in the cytoplasm in the form of numerous very small granules which increase in size. Fat functions as reserve food material in the production of energy, and when amoebae are starved the fat in the cytoplasm rapidly decreases.

Recent accounts of the effect of centrifuging on Protozoa have also been given by Miss R. Patten and H. W. Beams (1936), R. H. J. Brown (1936), and Miss Daniels (1938).

In Text-fig. 1 a and b are drawn from Brown's work on
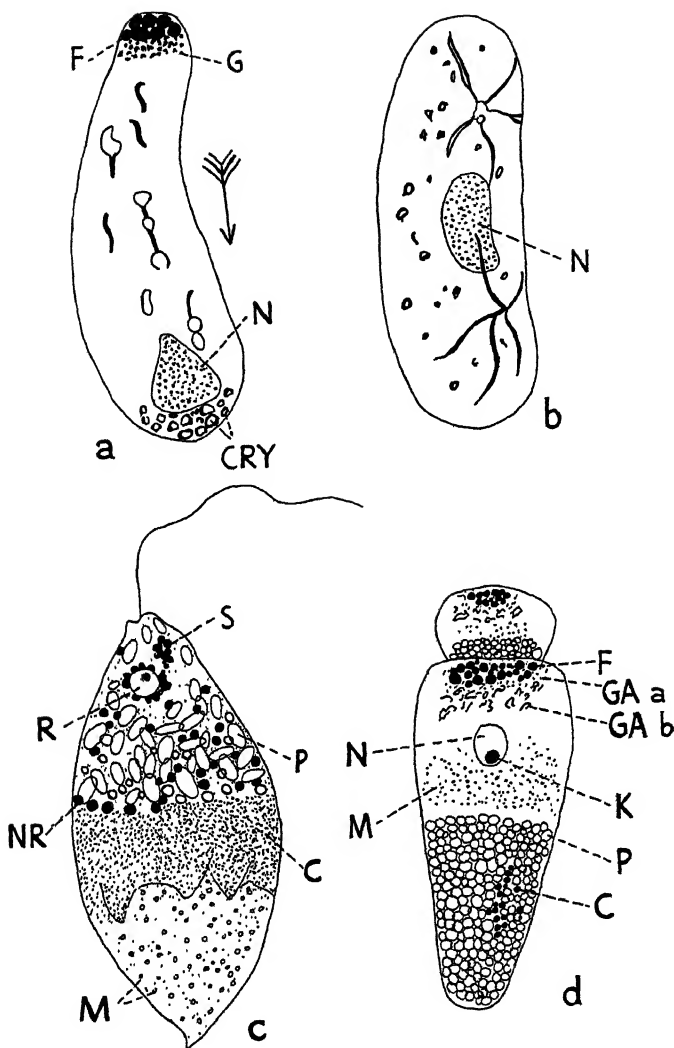
TEXT-FIG. 1.

Showing the effect of centrifuging on Protozoa. *a* and *b* from
Brown's work (1936). *N.*, nucleus; *Cry.*, crystals; *G.*, probably
rudimentary type of Golgi bodies; *F.*, fat. *c* From Patten and
Beams's work (1936). *S.*, stigma; *R.*, reservoir; *N.R.*, neutral-red
bodies; *P..* paramylum; *C.*, chloroplast; *M.*, probably mito-
chondria. *d* From Miss Daniels's work (1938). *F.*, fat;
G.Aa., Golgi granules; *G.Ab.*, Golgi apparatus; *N.*, nucleus;
K., karyosome; *M.*, mitochondria; *P.*, paraglycogen; *C.*,
chromidia.

Paramecium (unpublished), where he finds that in the centrifuged organism the nucleus and the crystals are moved towards the centrifugal side and the small granules that collect below the fat, at the centripetal end, are doubtfully of the nature of rudimentary Golgi bodies, which are revealed by both silver and osmic techniques.

Text-fig. 1 c is the modified diagram of Patten and Beams's work showing the effect of centrifuging Euglena. Here the chloroplast forms a belt having on the centrifugal side paramylum and neutral-red bodies, while clear cytoplasm containing probably mitochondria lies at the centripetal pole. They have also shown that the heaviest material in the organism may occupy either anterior, posterior, or lateral positions. As regards the Golgi bodies, they think that probably certain bodies that surround the reservoir resemble Golgi material, as they resist the action of bleaching after the osmic methods. In the case of Menoidium the paramylum and neutral-red staining bodies are the heaviest material, and in Chilomonas starch grains and neutral-red staining bodies occupy the centrifugal position. Neutral-red bodies were identified by them as being of the nature of volutin.

Text-fig. 1 d is from Miss Daniels's work showing the effect of centrifuging on the gregarines from the gut of Tenebrio larva. This diagram is especially interesting as it shows very clearly that fat and Golgi bodies occupy the centripetal position as is found in metazoan cells. Towards the centrifugal end is the nucleus, and below it is a layer of mitochondria followed by paraglycogen and chromidia. She found that neutral-red bodies are artefacts, and they remain scattered throughout the cell after centrifuging and staining with neutral red.

The problem was suggested to me by Professor J. Brontë Gatenby, to whom my thanks are due for help and criticism during my work.

2. MATERIAL AND METHODS.

The material used in this work consisted of Amoeba proteus 'Y'. Amoebae were cultured by the method described

by Sister Monica Taylor (1924). Wheat seeds were boiled to prevent germination, and two or more of these were put into each of the glass jars half-filled with tap-water at the room temperature. Some of the amoebae from the active growing culture were transferred to each of these jars, which were later on covered with glass plates and kept away from bright light.

For fixation and centrifuging, amoebae were collected in large quantities by the hand centrifuge. In all cases organisms were first studied in the living condition, with and without the help of vital dyes, before they were centrifuged.

The air-driven ultra-centrifuge was found too powerful for Amoeba proteus, so most of the work was carried out by means of the electrical centrifuge, which was used for $\frac{1}{2}$ hour to 1 hour at the speed of 5,000 revolutions per minute. Centrifuging was tried in culture solution as well as in thick gum arabic solution. In the latter medium the result was much better, and the organisms in most cases were not flattened out by sticking to the wall of the rotor as is the case when the culture solution is used as a medium for centrifuging. Fixatives were poured into the rotor or the centrifuge tubes immediately after centrifuging, in order to avoid the redistribution of the cytoplasmic bodies, which takes place very soon, due to the streaming movement of the protoplasm. Some of the amoebae were kept in the culture solution after ultra-centrifuging in order to study subsequent effects, when most of the cytoplasmic bodies like the crystals, nutritive spheres, contractile vacuole, fat, and in some cases nucleus, were thrown out of the cell.

For vital staining the National Aniline and Chemical Company's neutral red was used. It was tried in the watery solution as well as by Hall's alcoholic smear method (1929 b). In the former case the amoebae took more than an hour to stain properly, when a fairly strong solution of neutral red 1/10,000 was used. Hall's technique was found more satisfactory, and it stained the neutral-red bodies within 30–45 minutes. After staining with neutral red, 2 per cent. osmic acid was also introduced under the coverslip for varying lengths of time. Janus green B was used in the dilution of 1/30,000 to 1/40,000, and it took more than 2 hours to stain mitochondria. A 0·25

per cent. solution of Nile blue in normal salt solution gave a
very satisfactory test for fat and stained it deep blue within
30 minutes.

Schultz's recent method of staining fat, as described by Kay
and Whitehead (1935), proved quite satisfactory. Amoebae
were fixed in 10 per cent. formol for 10 minutes or more and then
they were placed in 50 per cent. alcohol for 5 minutes. They
were then transferred to the staining solution of Sudan IV for
30 minutes at the temperature of 37° C. The amoebae were
washed in 50 per cent. alcohol for a few seconds and then kept
in distilled water for a few minutes and were mounted in glycer-
ine jelly.

The Vitamin C test was tried as described in 'Microtomist's
Vade Mecum', 1937 edition. Five per cent. silver nitrate
solution was prepared, to 100 c.c. of which 5 c.c. of glacial
acetic acid were added. In this solution amoebae were kept
for 15 minutes or longer in the dark and then examined.

For glycogen both iodine and Best's carmine tests were tried,
together with hot water and saliva control. Both these methods
proved successful although the result was much superior in the
former case.

The fixatives employed were those of Da Fano, Cajal, Aoyama
('Microtomist's Vade Mecum', 1937 edition), Regaud, Champy,
Champy-Nassonov, Mann-Kopsch, Ludford, Bouin, and Schau-
dinn. In the case of Mann-Kopsch the time of post-osmication
was extended from 2–3 weeks. In Champy-Nassonov and Lud-
ford the post-osmication was carried out at 35° C., and the time
of post-osmication in the former case was 10–15 days and in
the latter 4–7 days. The Champy-Kull method of staining, acid
fuchsin and methyl green, and iron alum haematoxylin were
largely used. After Bouin or Schaudinn fixation, Delafield's
haematoxylin and light green combination of staining was also
used to stain nutritive spheres as mentioned by Taylor (1924).

Tests for lecithin, volutin, starch, and cholesterol as described
by Whitehead (1934) were also performed and will be described
where appropriate in the text.

3. Observations on Amoeba proteus 'Y'.

(i) General Morphology of Normal and Centrifuged Organisms.

In the endoplasm there is a single oval nucleus, a contractile vacuole, bipyramidal crystals, and nutritive spheres. Both nutritive spheres and crystals are evenly distributed throughout the endoplasm. In addition to these there are a large number of minute granules seen intra vitam. They were identified as mitochondria (Pl. 50, fig. 1).

When amoebae were stained supravitally with neutral red, a large number of rounded bodies varying in size took deep red stain and were called neutral-red bodies (Pl. 50, fig. 1). After Nile blue, Sudan IV and osmic methods a large number of fat-bodies are seen which are not very distinct intra vitam (Pls. 50 and 51, figs. 4, 5, 7, 9, 10).

Text-fig. 2 shows the stratification of the various cytoplasmic components, according to their specific gravity, after centrifuging. The amoebae become rounded after centrifuging, but sometimes they are flattened out when they stick to the wall of the rotor or the centrifuge tube. The cytoplasmic bodies when examined after centrifuging were found to be redistributed completely within a few minutes. This is due to the streaming motion of the protoplasm which causes these bodies to move, and thus the pseudopodia begin to be formed. After 10–15 minutes one can no longer make out whether the organisms were centrifuged or not. When the amoebae are ultra-centrifuged most of the cytoplasmic bodies are thrown out of the cell, and in such organisms the formation of pseudopodia does not take place for a long time. Some such amoebae were placed in the culture solution in order to study the after-effects of centrifuging. It was observed that they remain rounded for 10–15 days. It may be pointed out that observations on effects of ultra-centrifuging on the subsequent behaviour were not carried out, but it seems to me that they might lead to some interesting results.

A fact of importance may be pointed out here that although the amoebae remain rounded after ultra-centrifuging for a
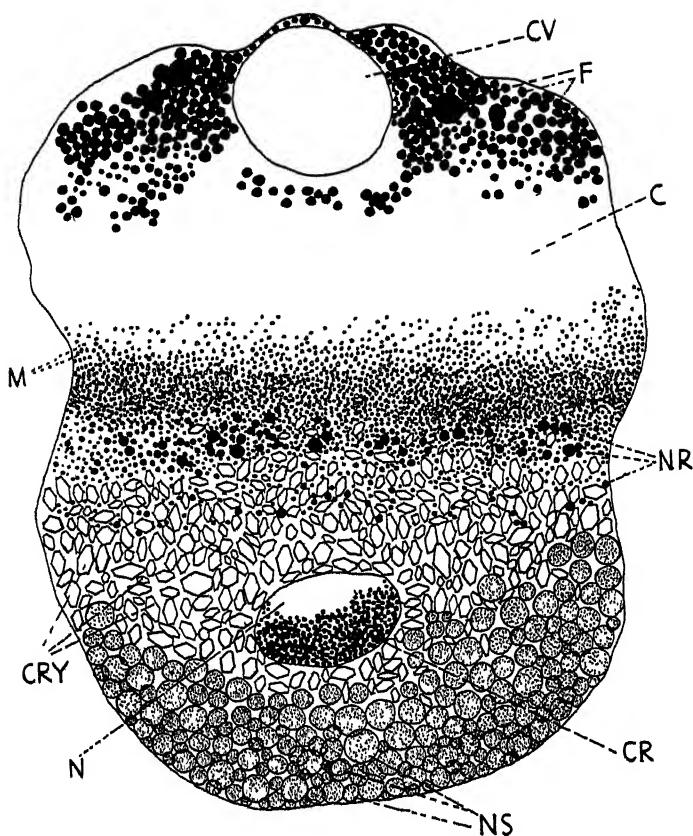
TEXT-FIG. 2.

Showing the stratification of the various cytoplasmic components
according to their specific gravity after centrifuging in Amoeba
proteus 'Y'.

considerable period and look as if they were encysted, yet I
could not observe any kind of special cyst wall surrounding
them.

(ii) Nutritive Spheres.

The nutritive spheres are numerous, rounded in shape, and
range from small to fairly big ones (Pl. 50, figs. 1, 4, 5; Pl. 51,

figs. 6, 7, 8, 9, 10). They contain glycogen which is revealed by both iodine and Best's carmine tests. They take a reddish brown colour after iodine and become red after Best's carmine. It may be pointed out that the former test was much more successful than the latter. After warm water and saliva control they do not take the proper colour by the above methods, although they do not disappear altogether. The nutritive spheres are stained a beautiful green after Champy fixation followed by acid fuchsin and methyl green (Pl. 51, fig. 6). Their shape remains as they are seen intra vitam. After Champy and iron alum haematoxylin they are not stained as a general rule, but sometimes the periphery of these spheres takes the haematoxylin stain. They are not revealed after Ludford, Champy-Nassonov, or Mann-Kopsch methods, but in most of the preparations spaces are very clearly seen indicating their position. Some of these spaces are drawn in Pl. 50, figs. 4 and 5, to distinguish them from fat, as the latter is very well seen after these methods.

A large number of irregular bodies lying in patches are revealed after the volutin test which stain a blue colour after methylene blue. This colour does not disappear even after keeping slides for 24 hours in 1 per cent. sulphuric acid. These bodies, in my opinion, are the same as the nutritive spheres, and they occupy the same position after centrifuging, as was seen in the case of nutritive spheres.

After Bouin or Schaudinn fixatives followed by Delafield's haematoxylin and light green, the nutritive spheres stain a light purple, but the periphery of these bodies takes a deep purple colour. When the time of counter-staining with light green is slightly increased they become colourless. In both these cases these bodies do not take the light green stain and remain in sharp contrast with the green colour of the general cytoplasm. They do not stain with any of the vital dyes used in connexion with this work, or with Sudan IV.

Text-fig. 2 and Pl. 51, figs. 8 and 10, show the effects of centrifuging on nutritive spheres. They occupy the extreme centrifugal position in the cell and are found to be the heaviest cytoplasmic component. Pl. 51, fig. 8, shows nutritive spheres after

centrifuging and staining with the iodine method for glycogen, when they take a reddish brown colour. When the ultra-centrifuge was used it was found that these bodies were thrown out of the cell. Such amoebae, when they were kept in the culture solution, remained rounded for a long time, and the nutritive spheres were reformed within 15 days or more.

I did not find the minute starch grains described by Sister Carmela Hayes (1924) by means of a solution of iodine in potassium iodide, nor could I see any kind of granule forming beautiful pictures around the large nutritive spheres as described by her in Amoeba proteus. No starch grains are present in these organisms, otherwise they would have been revealed after centrifuging and probably they would have occupied the centrifugal position in the cell as has been recently pointed out by Patten and Beams (1936) in Chilomonas after ultra-centrifuging.

I think that the nutritive spheres contain glycogen as a reserve food material. This fact is very clearly proved by studying the effects of ultra-centrifuging, in which case the amoebae remain rounded, appearing as if they were encysted until these bodies are formed. As soon as these bodies are formed the amoebae regain their normal shape and activity. When amoebae are centrifuged by the electrical centrifuge, in which case no cyto-plasmic bodies are thrown out of the cell, they resume their normal activity within a few minutes.

The term nutritive spheres for these bodies, as pointed out by Sister Monica Taylor (1924), seems to be more appropriate and correct than excretion spheres mentioned by Schaeffer (1916). The term excretion spheres is very misleading, and in no case should be applied to these bodies, because they are not the products of excretion but act as storage of food supply.

The glycogen is formed inside the spheres and is most probably secreted by the ground cytoplasm. Some amoebae contain large numbers of nutritive spheres, while others only a few, showing the amount of reserve food material present. It was also observed that the number of these spheres becomes less when the amoebae are starved.

(iii) Crystals.

The crystals in Amoeba proteus are numerous and vary in shape and size like the nutritive spheres described before. They are dissolved out by practically all the fixatives that were used in connexion with this work, and are not stained by any of the vital dyes. After centrifuging they collect towards the heavy end of the cell, and lie in between the nutritive spheres and neutral-red bodies (Text-fig. 2). In Amoeba proteus they do not occupy the extreme centrifugal position as pointed out by McClendon (1909), Harvey (1931), King and Beams (1934), and Brown (1936) in Paramecium. When the ultra-centrifuge is used the majority of the crystals are thrown out of the cell (Pl. 50, fig. 2).

The crystals are probably excretion products formed by the metabolic activity of the cell. In ultra-centrifuged organisms they are reformed after several days, when such amoebae are kept in the culture solution, and within 15 days or more they become as numerous as in uncentrifuged ones.

(iv) Neutral-red Staining Bodies.

As pointed out before, when amoebae are kept in 1/10,000 solution of neutral red a large number of bodies take a deep red colour after an hour, Pl. 50, fig. 1. By Hall's alcoholic smear method the staining is much quicker, and it was observed that within a few minutes some minute granules took the red stain. Later on the number of these granules increased, and within 30–45 minutes a large number of neutral-red bodies varying in size was revealed. Some of the neutral-red bodies were seen lying inside the food vacuoles. When 2 per cent. osmic acid was run under the coverslip, these bodies gradually lost their colour, and within 2–3 days all the neutral-red bodies became black due to the direct chemical action of neutral red and osmic acid, as has been pointed out by Hall and others in Protozoa. Some amoebae were also exposed to osmic vapour without previous staining with neutral red. In such organisms no blackening of the neutral-red bodies was seen. Also in Bouin and Schaudinn preparations followed by iron alum haematoxylin, neutral-red bodies are very clearly revealed, lying chiefly inside the food

vacuoles. It may be quite possible that the neutral-red bodies migrate into the food vacuoles and act as digestive enzymes in Amoeba proteus, as was observed by Volkonsky (1929) in the ciliates. I was unable to observe any definite relationship between the neutral-red bodies and the food vacuoles. The neutral-red bodies are never revealed by any of the Golgi techniques.

Some amoebae were centrifuged after staining with neutral red, and it was observed that the neutral-red bodies occupy the centrifugal position above the crystals (Text-fig. 2). When the ultra-centrifuge is used it is found that neutral-red bodies occupy the extreme centrifugal end because the majority of crystals and nutritive spheres are thrown out of the cell (Pl. 50, fig. 2). When the amoebae were first centrifuged and then stained by Hall's smear method, the same result was obtained. In this case the staining was successful within a few minutes, so it may be possible that this is due to the fact that all these bodies collect at one place. The above results lead to the conclusion that neutral-red bodies are of pre-existing nature in Amoeba proteus, and are not artefacts.   •

It may be pointed out that neutral-red staining creates some sort of poisonous effect on amoebae and they become rounded as was observed after centrifuging. The test for volutin was also performed, but I could not find that the neutral-red bodies were of the nature of volutin as was shown by Baker (1933) and recently confirmed by Patten and Beams (1936) in the case of free-living flagellates.

(v) Fat.

Fat is present in the form of a large number of rounded bodies. They are not very clearly seen intra vitam, but sometimes when the amoebae are kept too long in the solution of Janus green B, the fatty spheres are slightly stained greenish blue. By osmic technique of Mann-Kopsch, Champy-Nassonov, or Ludford, the fat-globules are very clearly seen (Pl. 50, figs. 4 and 5). They are more or less uniform in size and appear as solid spheres and not like spherules having dark rims. When using the high power of the microscope at a central focus they

look like solid bodies. After Champy fixative followed by either iron alum haematoxylin or acid fuchsin they are not stained, but sometimes they are seen in such preparations, being slightly osmicated. They are not revealed by any of the silver methods. By staining amoebae in Sudan IV, the fatty spheres take a deep red colour and the nutritive spheres remain colourless, and one can see both these types of bodies in fresh preparations (Pl. 51, fig. 9). It is rather interesting to note that by the Sudan IV method of staining, the fatty bodies are seen to vary in size, and some of them are as big as the nutritive spheres. This is probably due to the fact that osmic methods do not preserve all kinds of fatty substances. In Nile blue staining a very clear-cut picture, distinguishing between fatty spheres and nutritive spheres is obtained where the former type of bodies take the deep blue colour, while the nutritive spheres remain colourless (Pl. 51, fig. 7). Generally the fatty substances are stained by Nile blue within 20–25 minutes, but sometimes the time is a little longer. I was unable to identify the various fatty substances in Amoeba proteus, but I call these bodies Sudanophil fat as they are very nicely revealed by the Sudan IV method. No cholesterol was found to be present either inside the fatty spheres or separately.

After centrifuging, the fat-bodies occupy the extreme centripetal position in Amoeba proteus, and one can very clearly differentiate, by this method, fat from the nutritive spheres, as the latter collect at the extreme centrifugal side (Text-fig. 2). After centrifuging and then staining with Sudan IV it was observed that some of the fatty spheres cohere together and form big irregular lumps (Text-fig. 2 and Pl. 51, fig. 10). The fatty spheres are thrown out of the cell when the ultra-centrifuge is used, and when such organisms are stained with either Nile blue or Sudan IV these fatty bodies are not seen.

Pl. 50, fig. 5, shows the binary fission in Amoeba proteus. At this stage the fatty spheres are seen to be distributed more or less equally between the daughter cells.

As regards the formation of fat, I think it arises from the ground cytoplasm, and there seems to be no other source of fat in Amoeba proteus.

(vi) Nucleus.

As pointed out before, there is a single oval nucleus in Amoeba proteus. After centrifuging the nucleus moves towards the heavy end of the cell and lies between the crystals and the nutritive spheres. The chromatin, being the heaviest material inside the nucleus, collects towards the centrifugal end (Text-fig. 2). When amoebae are ultra-centrifuged the nucleus is thrown out from some of them. Such amoebae, it was observed, remain rounded for 3–4 days when placed in the culture solution, and being unable to elaborate food material, perish after several days.

(vii) Contractile Vacuole.

In Amoeba proteus there is a single rounded contractile vacuole. The wall of the vacuole never blackens with any of the osmic techniques even if the period of post-osmication is prolonged considerably.

After centrifuging, the contractile vacuole occupies the extreme centripetal position in the organism (Text-fig. 2, and Pl. 50 and 51, figs. 3 and 8). Sometimes the contractile vacuole is thrown out of the cell after ultra-centrifuging, and in some cases it was observed that several small vacuoles are to be seen lying at the centripetal end. It may be quite possible that the contractile vacuole is torn into several small vacuoles, which occupy the centripetal position. From the cells from which the contractile vacuole is thrown out, it was observed that it is reformed within several days when the organisms are kept in the cultural solution. I could not see the exact way in which it is formed, but it is certain that the contractile vacuole in Amoeba proteus is not a permanent structure as in Paramecium and other Protozoa

(viii) Mitochondria.

Mitochondria occur as small granules, which are very clearly seen intra vitam without any vital staining methods. After Champy fixative or Regaud followed by Champy-Kull staining, acid fuchsin and methyl green, or by iron alum haematoxylin, they are very well revealed, and take a deep red colour with acid

fuchsin and bluish grey to black after haematoxylin (Pl. 51, fig. 6). Sometimes in smear preparations with Bouin or Schaudinn, followed by iron alum haematoxylin, they are also seen, but their number is not so great as after mitochondrial fixatives. This indicates that an hour's fixation in Bouin or Schaudinn is not sufficient to dissolve out all the mitochondrial granules. After Ludford's modification of Mann-Kopsch, Champy-Nassonov, or Mann-Kopsch a large number of mitochondrial granules are seen to have taken a greyish black to black colour (Pl. 50, figs. 4 and 5). When such preparations are bleached with 0·5 per cent. potassium permanganate and 4 per cent. oxalic acid, the mitochondrial granules become grey and take the iron alum haematoxylin or acid fuchsin stain. These bodies are certainly mitochondria and should not be confused with other cytoplasmic bodies. Generally, with all mitochondrial fixatives and staining, a large number of rod-shaped bodies are also seen mostly lying in patches, and these were identified as bacteria and should not be confused with mitochondria. These rod-shaped bacteria are also seen after Bouin or Schaudinn followed by iron alum haematoxylin, and they lie mostly in patches inside the food vacuoles.

Janus green B was also used in various dilutions to stain mitochondria. When the concentration was too strong the nucleus and other cytoplasmic bodies stained deeply before the mitochondrial granules showed any sign of staining. It was found necessary to keep amoebae in a very dilute solution of Janus green 1/30,000 to 1/40,000 for hours. In one instance the mitochondrial granules took a blue stain within $1\frac{1}{2}$ hours (Pl. 50, fig. 3), but in many trials they did not stain at all. In certain cases some of the mitochondrial granules took the right stain while the rest of the granules remained unstained. The amoebae become rounded by keeping in Janus green solution, as was observed in the case of neutral-red staining.

It is interesting to note that the mitochondrial granules show Brownian movement in the living as well as in the dead cells. I observed that Brownian movement of these granules continued up to 5 or 6 days even when the amoebae were kept in 2 per cent. osmic acid for post-osmication.

After centrifuging the mitochondria occupy a position above the neutral-red bodies (Text-fig. 2).

Pl. 50, fig. 5, shows that during binary fission mitochondrial granules are distributed almost equally to the two daughter individuals. I could not observe the division of the mitochondrial granules either before or after binary fission.

(ix) Ectoplasmic Folds.

Ectoplasmic folds are seen after staining amoebae with Nile blue, Janus green, and in some cases with neutral red as well. They lie mostly on the surface, and when I saw them for the first time I thought that they might be a branched canalicular system, having definite walls, but later on I came to the conclusion that they are simply folds of the ectoplasm presenting a net-like arrangement. As far as I could make out they have no regular system of branching, but they are simply the prolongations of the ectoplasm, and where these prolongations meet they appear net-like. Sometimes these folds were seen to expand and break up at places, due to the streaming motion of the protoplasmic bodies. These folds are sometimes very clearly seen in ultra-centrifuged amoebae fixed by any of the silver or osmic methods. I am not sure of the function of these folds, but it may be quite possible that they give some kind of support to the organisms. As far as I know, no such folds have been observed by any of the previous workers in A m o e b a p r o t e u s. Since these folds were only seen in amoebae treated with dyes or fixatives, it may be possible that they are artificial.

(x) Vitamin C.

The test for Vitamin C was applied as described before. By this method a large number of minute granules are seen and they are mainly confined to the surface of the organism. Most of these granules lie in the ectoplasmic folds which are clearly seen by this method as well. These granules appear after 15 minutes or more, but they become slightly black after an hour. They look very much like the Vitamin C granules figured by various workers in different animals. I am somewhat doubtful as regards the pre-existing nature of these granules in A m o e b a

p r o t e u s , and they appear to be like silver precipitates formed
on the ectoplasmic folds and on the surface of the amoebae.
After centrifuging, these granules remain in the same position,
scattered throughout the organism, as was noted in uncentri-
fuged organisms. Such minute granules I also observed by
formalin-silver methods which were unaffected by centrifuging
and appeared to me like artefacts caused by silver precipitation.
No such bodies were seen by osmic methods.

Until further evidence is forthcoming, I am not fully con-
vinced as regards the validity of the silver nitrate method in the
demonstration of Vitamin C, judging by results in A m o e b a
p r o t e u s and other animal tissues examined by me.

4. DISCUSSION.

The observations on A m o e b a p r o t e u s show that the
cytoplasmic bodies are the sudanophil fat, a single contractile
vacuole, mitochondria, bipyramidal crystals, and nutritive
spheres. After centrifuging they stratify in different layers'
according to their specific gravity (Text-fig. 2). This clearly
shows that the supposed two types of Golgi bodies observed
by V. E. Brown (1930) do not exist in A m o e b a p r o t e u s .
My observations as far as the two types of bodies occurring
after Mann-Kopsch, Ludford's modification of Mann-Kopsch,
or Champy-Nassonov are in essential agreement with Brown's,
but the interpretation is altogether different. I never observed
at a central focus spherules appearing as rings or crescents; but,
on the contrary, I always found by the above methods round,
solid globules, which I call sudanophil fat, and not Golgi
bodies. These fat-globules are also revealed by Nile blue and
Sudan IV, and are more numerous and variable in size than
after osmic methods. I never observed these bodies after any
of the silver methods used to demonstrate Golgi bodies, and this
is a strong reason why they should not be called Golgi bodies.
Brown came to his conclusion without trying any of the silver
methods. The number of fatty spheres is much greater than
Brown has figured in his diagram. After centrifuging they
occupy the extreme centripetal position in the cell, as was noted
by Miss Daniels (1938) in Sporozoa. No mention has been made

by Sister Carmela Hayes (1924) of fatty spheres, although she tried the Sudan III and osmic tests.

In Pl. 50, fig. 2, Brown has drawn the same spherules having dark blue rims after Champy fixative and staining with acid fuchsin, thionin, and aurantia, which he observed after Mann-Kopsch. I never succeeded in staining fat-bodies by this method. I always succeeded in staining nutritive spheres deep green after Champy followed by acid fuchsin and methyl green. These bodies never show a deep green rim and light centres except when the staining is not good. I think in this case, perhaps, Brown figures as Golgi bodies what I consider to be nutritive spheres. Here again the number of such spheres drawn by Brown is few compared with what I have drawn in Pl. 51, fig. 6. The centrifuging clearly shows that the nutritive spheres and fat are two separate entities and should not be confused with each other. It is interesting to note that Sister Carmela Hayes (1924) has mentioned that in smear preparations the large nutritive spheres lose their spherical form and become irregular patches, which stain deeply in thionin and Delafield's haematoxylin. It seems possible that these are the same bodies that Brown calls Golgi bodies, after Champy fixation followed by acid fuchsin, thionin, and aurantia. It may be pointed out that the Champy method would never demonstrate Golgi apparatus except in germinal tissues.

Brown has also figured small granules of variable size taking a black colour after Mann-Kopsch and calls them Golgi bodies. I also succeeded in staining these granules by Mann-Kopsch, Champy-Nassonov, and Ludford's method, but I call them mitochondria. After bleaching they become grey and are stained by iron alum haematoxylin or acid fuchsin. These mitochondrial granules are revealed by both Champy and Regaud fixatives stained with iron alum haematoxylin or acid fuchsin. They are also stained with Janus green B. The number of such granules figured by Brown is certainly less than I find after Mann-Kopsch and other fixatives. According to Brown, these small granules, which I call mitochondria, transform into spherules with dark rims. I never observed any relationship existing between mitochondria and fat, or mitochondria and

nutritive spheres. Certainly both fat and mitochondria are clearly seen after Mann-Kopsch. I was never able to make out small spherules attached either to the fat-globules or to the nutritive spheres as noted by Brown.

In Amoeba proteüs the wall of the contractile vacuole never blackens after any of the osmic methods, even if the period of post-osmication is prolonged considerably. This is in accord with the findings of Brown.

Among Sarcodina, Hall (1930 b) in Amoebida, Hall and Loefer (1930), Nigrelli and Hall (1930) in Testacea, have claimed that the neutral-red bodies are the homologue of the metazoan Golgi apparatus. They say that these bodies are blackened when exposed to osmic vapour after staining with neutral red, and are also revealed by osmic and silver methods used for the demonstration of the Golgi apparatus. I certainly do not agree with them, because we have never seen Golgi bodies stained with neutral red either in Protozoa or in Metazoa, except when the cells are dead. In Amoeba proteus I always succeeded in staining certain pre-existing bodies with neutral red, and these are certainly blackened when amoebae are first stained with neutral red and then exposed to osmic vapour, but when amoebae are exposed to osmic vapour without previously staining with neutral red, these bodies do not blacken. This shows that there is some kind of chemical action between neutral red and osmic acid by which these bodies are blackened, and this has no cytological significance. For further details as regards neutral-red cytology, see Douglas, Duthie and Gatenby (1933). I never observed the blackening of the neutral-red bodies by either silver or osmic methods used for the demonstration of the Golgi apparatus. The fact that neutral-red bodies lying inside food vacuoles are revealed by Bouin or Schaudinn followed by iron alum haematoxylin clearly shows that they are not the homologue of the Golgi apparatus. The effect of centrifuging shows that the neutral-red bodies occupy the heavy end of the cells, which is another source of evidence that they cannot be homologized with the Golgi apparatus. Miss Daniels has shown (1938) that in Sporozoa the Golgi bodies occupy the centripetal position, as has been noted in metazoan cells.

Gatenby (1937) says: 'Neutral red is an important negative test for the Golgi apparatus. If some body or area stains in neutral red this is a certain test that it is not Golgi material. Red globules often appear inside the Golgi apparatus and thus mark its position.' My results in Amoeba proteus are in agreement with this.

Causey (1925) in Entamoeba gingivalis has shown supposed Golgi bodies as net-like in appearance. He fixed smears in osmic vapour, and stained with Regaud Fe-haematoxylin or Bensley's acid fuchsin and methyl green. I did not observe any such network in connexion with food vacuoles in Amoeba proteus as was noted by Causey. According to King (1927), and Bowen (1928), the technique used by Causey is inadequate for the demonstration of the Golgi apparatus, and his work needs confirmation by appropriate methods.

Hirschler (1927a) in Entamoeba blattae figures the typical sporozoan type of Golgi bodies, and he was able to impregnate them both with osmic and silver methods. This conclusion will also need confirmation before it can be accepted.

Finally, some remarks may be made on the interesting work of Mast and Doyle (1935a and 1935b).[1] It is very clear from my observations that these workers have probably studied the cytoplasmic bodies of an altogether different species of amoeba, or of a different variety of Amoeba proteus. There are, of course, well-marked varieties of Amoeba proteus, one variant characterized by an entire absence of crystals, other varieties due to the size of the nutritive spheres, &c. It may be pointed out that the differences may be also due to the food supply and temperature.

[1] I am indebted to Dr. Doyle for a personal communication, in which he has pointed out that the Amoeba proteus which he used in connexion with his work with Dr. Mast at Johns Hopkins University is quite different from the one I have used in the present investigation. As a result of his brief study of the specimens obtained from Sister Monica Taylor, he agrees with me that the nutritive spheres (refractive bodies of Mast and Doyle) in this case stain red with iodine. He has also pointed out that crystal vacuoles are present around all crystals and occasional blebs are seen. I must emphasize that I never saw either crystal vacuoles or blebs in my specimens of Amoeba proteus.

The Amoeba proteus on which I have worked never contained plate-like crystals. I have always seen only bipyramidal crystals as was pointed out by Schaeffer (1916) in the case of Amoeba proteus. These crystals lie free in the cytoplasm and never inside crystal vacuoles. Brownian movement of the crystals is well marked. I have not seen alpha granules intra vitam, but these granules may be the same as Vitamin C granules described by me, although I doubt the pre-existing nature of the latter type of granules. The above facts are made clear by comparing my Text-fig. 2 with Mast and Doyle's Fig. 1 A. The stratification of the cytoplasmic bodies after centrifuging is quite different from the results of these workers, except in the case of fat, contractile vacuole, chromatin of the nucleus, and the nutritive spheres. I think that some of these differences are probably due to the fact that they have applied low pressures while centrifuging, in which case the stratification is not very clear cut.

I found a large number of pre-existing neutral-red staining bodies, which are quite separate from the rest of the cytoplasmic bodies, and these occupy the position in between the mitochondria and the bipyramidal crystals after centrifuging. These bodies were either confused by these workers with some other cytoplasmic bodies or they do not occur in the Amoeba proteus described by them. I think that the elongation of the amoebae up to the length indicated in the Fig. 1 A of Mast and Doyle is due to the fact that the organisms stick to the wall of the rotor or the centrifuge tube during centrifuging, otherwise they remain perfectly rounded. The fat-globules are thrown out of the cell by using the ultra-centrifuge, but not in the way described by Mast and Doyle, who point out that the fat-bodies form aggregations in the pseudopodia, and these are later on separated from the rest of the body.

The shape of the mitochondria is always granular, either in the fixed or in the living amoebae. I never observed that these granules change in form and become rod-like or ellipsoidal. I could not see the regular distribution of the mitochondria over the contractile vacuole either in the living or in the centrifuged organisms, and thus I am not prepared to accept the view that

mitochondria function in excretion as pointed out by Mast and Doyle. The wall of the contractile vacuole never blackens with osmic acid even after prolonged periods of osmication, and therefore Nassonov's (1924) hypothesis cannot be accepted in this particular case.

Mast and Doyle's work in proving that mitochondria are essential for the life of the animal is certainly very interesting. I do not agree with these workers that mitochondria function in transporting the enzymes to the food vacuoles and in transferring substances from the food vacuoles to the cytoplasm. The movement of the mitochondria near the food vacuoles, studied by me, does not give any idea that mitochondria play such a rôle in the life of the organism. I think that the neutral-red bodies which are present in the food vacuoles function as digestive enzymes.

I have not observed that there is any relation between the crystals and the nutritive spheres. The substance of the crystals is not used up in the formation of nutritive spheres. I think that the crystals are excretory products present in the cytoplasm and are formed in large numbers when they are thrown out of the cell by using the ultra-centrifuge. As no crystal blebs are present in Amoeba proteus, the idea of Mast and Doyle, that the blebs are formed from the vacuole refractive bodies and give rise to the outer layer of refractive bodies, is not convincing.

I was not able to make out vacuole refractive bodies except the neutral-red bodies lying inside the food vacuoles, and they are quite similar to the neutral-red bodies present in the cytoplasm. They are seen after Bouin and iron alum haematoxylin, lying mainly in food vacuoles, but they are never seen after the osmic methods, as was pointed out by Mast and Doyle. These facts clearly rule out the possibility that they are Parat's or Hall's vacuome, as supposed by these workers. There is no evidence to show that the substance of these bodies is used up in the formation of nutritive spheres.

I prefer to call the refractive bodies nutritive spheres, because they show the reaction of glycogen, as pointed out by Sister Carmela Hayes and confirmed by me. I never observed that

the outer portion of the nutritive spheres gives positive Golgi tests, as was recorded by Mast and Doyle. I am sure that with silver methods used for Golgi apparatus, these spheres are never seen, and with osmic methods spaces remain behind indicating their presence, as I have shown in Pl. 50, figs. 4, 5. I have already pointed out that these bodies can never be called Golgi bodies or Golgi substance. By keeping amoebae for 2–3 days in neutral-red solution, these bodies are stained red, but the two parts (as indicated by Mast and Doyle) were not very clear, although in some cases I could see outer and inner areas. In fixed or stained preparations I was not able to note these two parts.

I do not altogether agree with the interpretation of these workers that Sister Monica Taylor (1924) has identified nutritive spheres to be stages of the encysted amoebae, because she was able to identify these two structures quite separately, and according to her these encysted forms do not occur throughout the year. I must confess that I was not able to see the encysted amoebae during the period I was working, and moreover, I was not particularly interested at that moment in studying the life-cycle of these organisms. I agree with Mast and Doyle that the nutritive spheres are reserve food material, and their number is decreased when the amoebae are starved. These bodies are never stained with Nile blue sulphate.

The fat-globules present in the cytoplasm always stain deep blue with Nile blue sulphate, and never pink as was pointed out by Mast and Doyle. Sometimes by this method I found fat-globules lying inside the food vacuoles, but their reaction was similar to those of the fat-globules present in the cytoplasm, so I do not support the view of Mast and Doyle that the fat-globules lying outside and inside the food vacuoles have a different chemical composition. I think that the fat-globules are secreted by the ground cytoplasm. It may be possible that some of the fat-globules come from Chilomonads eaten by the amoebae, but I am not prepared to accept the view that all the fat-globules present in the cytoplasm come from the Chilomonads, as claimed by Mast and Doyle.

Until more work has been done in the Sarcodina group of

Protozoa, I am not prepared to homologize any structure in Amoeba proteus with either metazoan or sporozoan Golgi apparatus, and I certainly do not agree with Hall and others who claim that neutral-red staining bodies are the homologue of the Golgi apparatus.

5. SUMMARY.

1. When Amoeba proteus is subjected to high centrifugal force most of the cytoplasmic bodies are thrown out of the cell, so this work was done with the ordinary electrical centrifuge.

2. The stratification of the various cytoplasmic components according to their specific gravity is as follows: the contractile vacuole and the fat, being the lightest, occupy the centripetal position; then there is a layer of cytoplasm followed by mitochondria, neutral-red bodies, crystals, and nutritive spheres. The nucleus occupies a position in between the crystals and the nutritive spheres (Text-fig. 2).

3. The redistribution of the various cytoplasmic components takes place within a few minutes after amoebae have been centrifuged by the electrical centrifuge. Ultra-centrifuged organisms kept in culture solution remain rounded for 10–15 days, and no cyst formation takes place. The crystals and nutritive spheres are reformed; the former seem to be the products of excretion formed by the metabolic activity of the cell.

4. The nutritive spheres contain glycogen as reserve food material, and give positive tests for glycogen with iodine and Best's carmine.

5. There is no evidence that the bodies which stain with neutral red are the homologue of the metazoan Golgi apparatus, although they are pre-existing bodies in Amoeba proteus. The contractile vacuole does not blacken even after prolonged osmication. No certain homologue of the Golgi apparatus was found in Amoeba proteus.

6. Fat and glycogen are two distinct types of storage material present in Amoeba proteus. The former is very well seen with osmic acid, Sudan IV, and Nile blue tests.

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DESCRIPTION OF PLATES 50 AND 51.

LETTERING.

F., fat; *C.V.*, contractile vacuole; *C.*, cytoplasm; *M.*, mitochondria; *N.R.*, neutral-red staining bodies; *N.*, nucleus; *C.R.*, chromatin; *CRY.*, crystals; *N.S.*, nutritive spheres; *F.V.*, food vacuole.

PLATE 50.

Fig. 1.—Living Amoeba proteus stained with neutral red; uncentrifuged.

Fig. 2.—As for fig. 1, but ultra-centrifuged, showing the stratification of cytoplasmic bodies when the majority of the crystals and nutritive spheres are thrown out of the cell. Fat-bodies are not seen.

Fig. 3.—Stained with Janus green B, uncentrifuged.

Fig. 4.—Mann-Kopsch preparation, unstained and uncentrifuged.

Fig. 5.—Champy-Nassonov preparation, unstained, showing the last stage of binary fission, uncentrifuged.

PLATE 51.

Fig. 6.—Champy preparation stained with acid fuchsin and methyl green. Mitochondria are red and nutritive spheres deep green, uncentrifuged.

Fig. 7.—Nile blue staining, uncentrifuged. The fat-bodies have taken deep blue colour while the crystals and the nutritive spheres are colourless.

Fig. 8.—Iodine test for glycogen, centrifuged. The nutritive spheres take reddish brown colour.

Fig. 9.—Sudan IV staining, uncentrifuged.

Fig. 10.—Sudan IV staining, centrifuged.

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